TESIS DOCTORAL

Caracterización de material de dragado optimizando un método integrado de evaluación de la calidad ambiental

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CARACTERIZACIÓN DE MATERIAL DE DRAGADO OPTIMIZANDO UN MÉTODO INTEGRADO DE EVALUACIÓN DE LA CALIDAD AMBIENTAL

M.Carmen Casado Martínez

Cádiz, 2006

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(M.Carmen Casado Martínez)

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HACEN CONSTAR:

Que esta memoria, titulada "**Caracterización de material de dragado de puertos Españoles optimizando un método integrado de la calidad ambiental**", presentada por Dña M.Carmen Casado Martínez, resume su trabajo de Tesis Doctoral y, considerando que reúne todos los requisitos legales, autorizan su presentación y defensa para optar al grado de Doctor en Ciencias del Mar por la Universidad de Cádiz.

Cádiz, Junio de 2006

Dr. T.Ángel DelValls Casillas

Dr. Jesús M. Forja Pajares

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"A la vida, esa gran obstinada"

Índice de Contenidos

Índice de Abreviaturas

BAF	Siglas inglesas para el factor de bioacumulación.
BSAF	Siglas inglesas para el factor de acumulación biota/sedimento.
CEDEX	Centro de Estudios y Experimentación.
EqP	Siglas inglesas para denominar los coeficientes de partición.
ERL/ERM	Siglas inglesas para las guías de calidad de sedimentos desarrolladas por la NOAA.
GIPME	Siglas inglesas para el grupo de expertos en polución en el medio marino.
IMO	Siglas inglesas para la Organización Marítima Internacional.
LC	Siglas inglesas para la Convención de Londres.
MARPOL	Siglas inglesas para la Convención para prevenir la polución en el medio marino.
NA1	Nivel de acción inferior de las RRGMD.
NA2	Nivel de acción superior de las RRGMD.
NNAA	Niveles de Acción.
NOAA	Siglas inglesas para la Asociación estadounidense para la atmósfera y los océanos.
OSPAR	Siglas inglesas para designar la Convención de Oslo y París.

PAHs	Siglas inglesas para los hidrocarburos policíclicos aromáticos.
PCBs	Siglas inglesas para los bifenilos policlorados.
PIANC	Siglas inglesas para la Asociación internacional permanente para la navegación y el comercio.
RRGMD	Recomendaciones para la gestión de material de dragado.
SQGs	Siglas inglesas para las guías de calidad de sedimento.
TBP	Siglas inglesas para denominar el potencial de bioaccumulación teórico calculado mediante los factores de bioacumulación medios –BSAF-.
US EPA	Siglas inglesas para la agencia ambiental de los Estados Unidos.
WFD	Siglas inglesas para la nueva Directiva Marco del Agua.

Capítulo 1.

Introducción, objetivos, zonas de estudio y organización de la tesis

1. Introducción

En los dos últimos siglos una gran proporción de la población mundial se localiza en una estrecha franja litoral. La bondad de las condiciones climáticas respecto a un riguroso interior y en especial un mayor desarrollo económico han motivado el movimiento masivo de la población a la zona costera. La navegación marítima ha jugado un papel fundamental como desencadenante de este crecimiento económico mediante el establecimiento de importantes rutas comerciales a lo largo de la historia y, posteriormente, mediante la localización de lugares considerados estratégicos para el movimiento de pasajeros y mercancías donde se efectúan actividades comerciales e industriales de importancia local, nacional e internacional. En España sólo la red de Puertos del Estado tiene previsto un crecimiento del transporte de mercancías de más del 22% para el 2006 en relación a los valores del año 2000 gracias al incremento del tráfico marítimo como alternativa en transportes de corta distancia y se estiman en casi 150.000 el número de puestos de trabajo generados directa o indirectamente (www.puertosdelestado.es). Paralelamente las zonas portuarias en sí, y toda la estructura social y económica establecida en los alrededores, han influido en mayor o menor medida sobre los aspectos ambientales de los diferentes ecosistemas. Pueden producirse efectos de tipo visual, acústicos, vibraciones, así como una disminución de la calidad del aire y otros impactos relacionados con el iluminado de la zona portuaria (PIANC, 1997). Además, y como resultado de políticas poco protectivas con el

medio ambiente, se han venido vertiendo todo tipo de desechos (los producidos en tierra así como los producidos por las embarcaciones) que pueden inducir cambios en los recursos naturales y en general disminuir la calidad de las aguas y del sedimento.

Consideraciones ambientales de los materiales de dragado

Una de las áreas que concentra mayor interés ambiental es la calidad del agua y del sedimento, o lo que es lo mismo, la contaminación marina. Desde el punto de vista de los ecosistemas marinos se pueden distinguir dos tipos distintos de actividades que ejercen una fuerte presión sobre ellos:

- Actividades indirectas al puerto, como pueden ser las actividades del conjunto de industrias que suelen rodear el área portuaria o están físicamente dentro de ella, ya que en muchos casos suelen verter directamente lodo industrial o lodos de tratamiento. Además estas zonas han sufrido el vertido de residuos urbanos, tanto de tipo doméstico, pluvial e industrial, que en algunos casos continúa aún hoy en día.
- Actividades directamente relacionadas con el puerto, como el trasiego de pasajeros y mercancías, carga y descarga, obras portuarias, etc.

Tanto las actividades directas como las indirectas van a introducir presiones al medio principalmente en forma de vertidos de muy distinta naturaleza y peligrosidad, muchas veces sin tratamiento previo. Estos vertidos pueden producirse de forma accidental, de forma ilegal o bien pueden tener su origen en el desarrollo operacional del puerto (descargas de barcos, residuos peligrosos y de carácter industrial, lodos y basuras, derrames tanto de petróleo como de otros productos químicos y materiales de dragado). Como consecuencia del origen multi-sectorial de la contaminación, los puertos se van a ver afectados, en mayor o menor medida, por una variedad de contaminantes ambientales. Se han clasificado en tres grupos:

- Organismos patogénicos, de especial importancia en zonas donde se han vertido aguas residuales.
- Macrocontaminantes, como por ejemplo nutrientes tipo fosfatos o nitratos o material en suspensión que puede afectar a la turbidez.
- Microcontaminantes, generalmente en concentraciones bajas pero que pueden introducir efectos nocivos y producir una alteración del sistema. Se incluyen en este

grupo los metales traza, sustancias inorgánicas (p.e. cianuros), hidrocarburos, hidrocarburos aromáticos, hidrocarburos policíclicos aromáticos, hidrocarburos clorados (p.e. PCBs) y los pesticidas.

Las actividades de dragado en zonas portuarias y vías de navegación se han realizado de forma rutinaria para la construcción de nuevas infraestructuras portuarias y para el mantenimiento de la navegación desde tiempos inmemoriales ya que la mayoría de puertos presentan zonas de baja movilidad donde la sedimentación es muy probable. En las últimas décadas se realizan de forma rutinaria grandes obras de dragado para aumentar el calado de puertos y canales, y satisfacer así las necesidades de buques de gran tamaño. Los efectos de estas actividades en el medio ambiente y en la salud humana no se habían tenido en cuenta pero la alteración evidente del ecosistema marino y algunos accidentes producidos por el vertido de sedimentos contaminados han cuestionado su salubridad y, si bien su realización no se cuestiona por considerarse fundamental en el crecimiento económico del puerto, las grandes obras de dragado han sido cuestionadas en los sectores ambiental, social y económico. Por cuestiones técnicas y/o económicas la mayoría de estos sedimentos va a ser finalmente vertida en el mar, idealmente en la misma zona de dragado y mantener así el sistema en equilibrio. Si los materiales de dragado resultan inocuos se pueden incluso considerar un recurso valioso y pueden usarse por ejemplo en la regeneración de playas. El vertido al mar de materiales de dragado puede producir efectos indeseables debido a la resuspensión y sedimentación de grandes volúmenes de sedimento, causando gran turbidez y produciendo la muerte o el aplastamiento de la fauna bentónica, y a largo plazo alteraciones en la textura del fondo y en las condiciones hidrodinámicas de la zona.

Una de las cuestiones que ha despertado un interés medioambiental es el vertido de sedimentos contaminados, debido a la capacidad destructora que puede tener en el medio receptor. Los sedimentos actúan como sumidero de un gran número de sustancias indeseables pero, si se dan las condiciones físicas, geoquímicas y biológicas necesarias, pueden convertirse en fuentes de contaminación y producir modificaciones en la diversidad de las comunidades bentónicas o incluso efectos acumulativos de los contaminantes a través de la cadena trófica. Tres son las vías a través de las cuales estas sustancias (metales, metaloides, aceites y grasas, hidrocarburos y otros derivados del petróleo, pesticidas, etc.) pueden causar efectos adversos en los organismos: 1) por interacción con la fase líquida, es decir, a través del agua intersticial o por contacto con las aguas de mezcla producidas en la resuspensión del sedimento, 2) por contacto directo con el sedimento, ya sea por ingestión o bien por el carácter bentónico de los organismos, y 3) a través de la cadena alimenticia;

los efectos que van a producir dependen en gran medida de la especiación química del contaminante, tanto en el agua como en el sedimento, ya que va a modificar la capacidad de interacción con los tejidos y causar efectos en el organismo en sí y en sus consumidores, incluyendo el ser humano (Neff, 2002).

La gestión de dragados portuarios en España

Situación legislativa y contexto internacional

En los últimos años ha aumentado considerablemente la sensibilización social por la conservación del medio ambiente y, en general, por el desarrollo de políticas dirigidas a la conservación y uso sostenible de los recursos naturales. Esto se ha visto reflejado en numerosos convenios para prevenir efectos adversos en el medio ambiente, unos destinados al control de las fuentes y otros a la conservación de determinados hábitats de especial interés. Entre las primeras destacan el Convenio sobre la Conservación de la Biodiversidad (Río de Janeiro, 1992) y el Convenio Ramsar (Convenio sobre humedales de importancia internacional, Ramsar, 1971). Por otro lado son de aplicación el Convenio para la prevención de la contaminación marina por vertido de residuos y otras materias (Londres, 1972), el convenio internacional para la prevención de la contaminación de embarcaciones (MARPOL, 1973/78), el Convenio para el control de movimientos transfronterizos de residuos peligrosos y su vertido (Convención de Basilea, 1989), y a nivel regional, la Convención para la protección del ambiente marino del Noreste Atlántico (Convención OSPAR, 1992).

En la práctica, estos convenios han permitido la introducción de políticas más protectoras con el medio ambiente en los países firmantes, y en consecuencia se ha establecido un control a nivel legislativo de las actividades que puedan suponer un riesgo para el medio. La Organización Marítima Internacional (IMO, 1998) estima entre 200 y 300 millones de m3 el volumen de materiales dragados vertidos anualmente al mar. El vertido de estos materiales de dragado y otros residuos en el mar está regulado a nivel internacional por el Convenio de Londres (1972), el Convenio OSPAR para los países firmantes del Noreste Atlántico y del Mar del Norte, el Convenio de Barcelona (1976) para los del Mediterráneo o el Convenio de Helsinki (1992). Estos convenios controlan el vertido mediante un sistema de permisos que sólo pueden ser obtenidos una vez estudiado el

impacto potencial que podría introducir el material de dragado en el medio marino en caso de aprobar su vertido la mar.

Hoy en día, la mayoría de países regulan las actividades de dragado y su posterior vertido para cumplir con las recomendaciones internacionales como firmantes de estas iniciativas. Más de 70 países han aceptado los principios del tratado en materia de prevención de la contaminación bajo el Convenio de Londres (IMO, 1982) y actualmente están trabajando para incorporar el nuevo Protocolo de 1996, que incluye nuevos componentes en materia de gestión y regulación. La Declaración de 1972 incluía no sólo la lista de materias y compuestos que no pueden ser vertidos al mar (Anejo I -LC, 1972-) sino que asentaba las bases para la puesta en marcha de un sistema de permisos para el vertidos al mar en función de la presencia de determinados contaminantes (Anejo II –LC, 1972). El nuevo Protocolo de 1996 sigue la línea anterior manteniendo los principios de precaución y prevención, impulsando firmemente la colaboración inter-sectorial para resolver enfrentamientos y llegar a la solución ambiental, social y económica más deseable. Sólo en Europa, el coste de gestión de este tipo de materiales se ha estimado en más de un billón de euros al año.

Las "Recomendaciones para la Caracterización de Material de Dragado" (CEDEX, 1994).

En España, el Convenio de Londres se recoge en la legislación en la Orden de 26 de Mayo de 1976. Aunque no existen herramientas a nivel legislativo, la normativa se plasma en las "Recomendaciones para la Caracterización de Material de Dragado" (CEDEX, 1994), cuya aplicación es acordada en Mayo de 1994 por las Administraciones implicadas en este tema y que se han venido utilizando de forma regular desde entonces. Estas recomendaciones fueron redactadas por el grupo de autoridades representantes de los distintos sectores de interés y, en general, siguen las guías establecidas por el Convenio de Londres para el vertido de material de dragado (IMO, 1998), así como otros convenios y tratados que afectan a España a nivel regional (OSPAR, Barcelona, Helsinki).

Aunque estas recomendaciones (RRGMD) contemplan como primera opción el reciclado de este tipo de materiales, están fundamentalmente enfocadas a la regulación del vertido de materiales de dragado al medio marino en función de su calidad y son de aplicación siempre, ya sea necesaria la declaración de impacto ambiental o no. De forma resumida estas recomendaciones utilizan una serie de guías químicas para la evaluación de

la calidad de sedimentos que son comparadas con los resultados de los análisis químicos y permiten autorizar o no el vertido, aunque actualmente están en proceso de revisión.

Los estudios requeridos para la gestión de los materiales en las RRGMD incluyen un resumen del proyecto y de las características de la zona a dragar, la caracterización de los sedimentos y un estudio comparativo de las distintas alternativas de vertido. El procedimiento para la autorización del vertido al mar de dragados portuarios incluido en las RRGMD (Figura 1.1) tiene como punto de inicio la caracterización del proyecto y de la zona a dragar (tipo y duración del dragado, técnicas de extracción y vertido de los materiales, definición de la zona a dragar, etc.) y la revisión de toda información disponible que pueda ser útil para autorizar o no el vertido al mar. Si el dragado es de pequeña envergadura o si está compuesto fundamentalmente por arenas o gravas, no sería necesaria una mayor caracterización de los sedimentos para obtener el permiso de vertido al mar.

También las evidencias de fuentes directas o difusas de contaminación en la zona pueden ser suficientes para decidir que el vertido al mar no es la opción más adecuada, y en este caso no sería necesaria una mayor caracterización de los sedimentos para su posterior gestión. Cuando no se dispone de información suficiente para la toma de decisiones, se deberán llevar a cabo los estudios necesarios para poder establecer si el vertido al mar puede ser autorizado. Para simplificar la gestión y agilizar el sistema de permisos necesarios según estas recomendaciones se establecen tres categorías de materiales de dragado "en función de los efectos de naturaleza química y/o biológica que pueden producir en la biota marina":

- Categoría I para aquellos materiales cuyas concentraciones de contaminantes son bajas o cuyos efectos bioquímicos en la flora y fauna son nulos o prácticamente insignificantes. Para estos materiales de dragado se permite el vertido libre al mar, aunque considerando los posibles efectos mecánicos.
- Categoría II para los materiales con concentraciones moderadas de contaminantes. Estos pueden ser vertidos de forma controlada previa autorización especial, y en todos los casos se debe llevar a cabo una adecuada selección de la zona de vertido en función del impacto y un programa de vigilancia ambiental siguiendo las indicaciones incluidas en las citadas Recomendaciones.
- Categoría III para los materiales con concentraciones elevadas de contaminantes. Estos materiales deben ser aislados o tratados adecuadamente según las indicaciones incluidas en las RRGMD. Dentro de ésta se pueden distinguir dos

subcategorías en función del grado de contaminación y que van a determinar el grado de aislamiento necesario. Este tipo de medidas de aislamiento pueden ser de tipo blando o duro según permitan o no el intercambio de las aguas lixiviadas con el medio receptor.

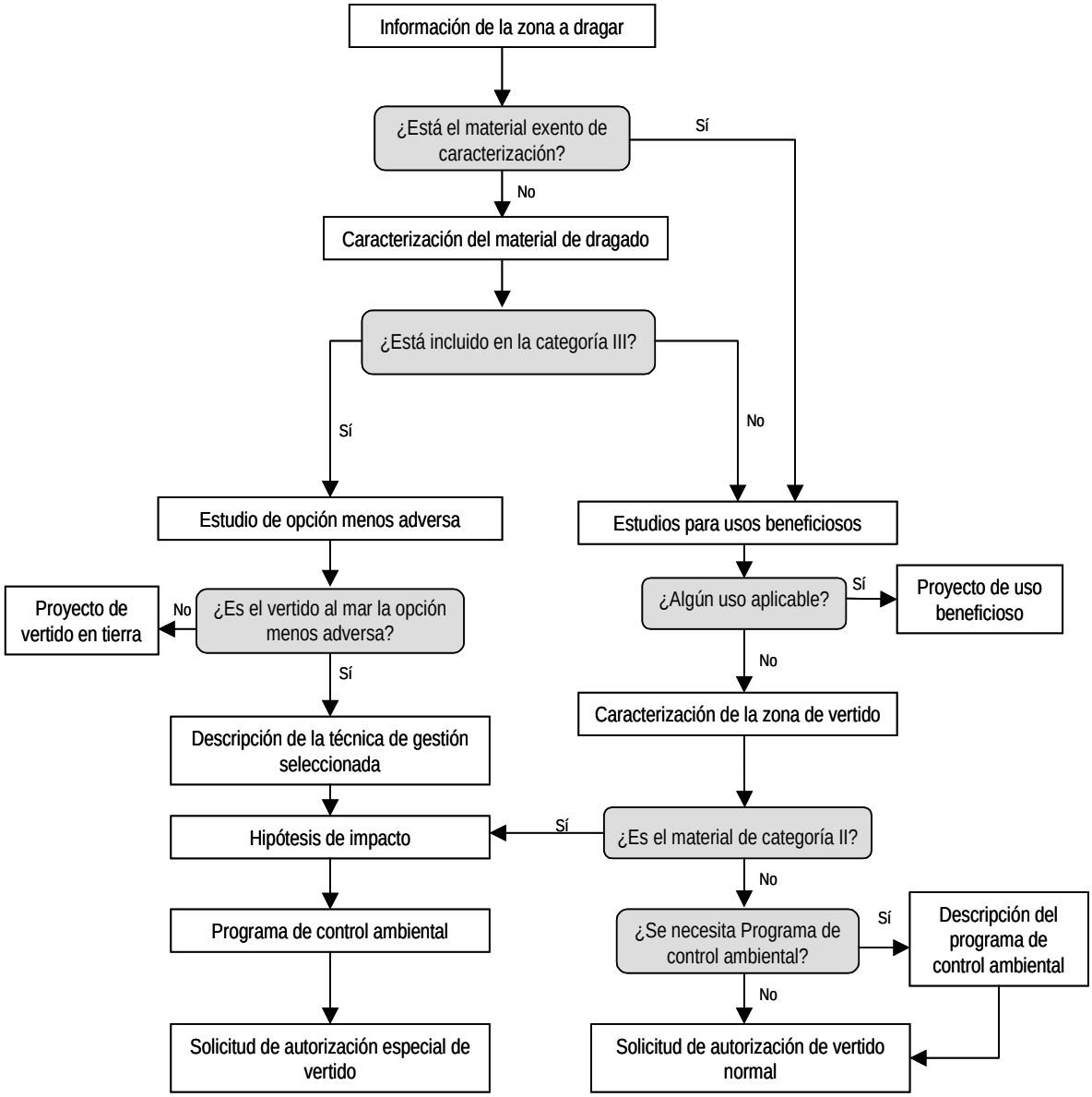

Fig. 1.1. Procedimiento para la gestión de materiales de dragado en España (CEDEX, 1994).

Las RRGMD incluyen las guías técnicas para la selección de las estaciones de muestreo así como para la caracterización de los sedimentos. En la práctica, hasta el momento se han venido utilizando los denominados Niveles de Acción (NNAA), definidos como las concentraciones de sustancias tóxicas que son utilizadas para la clasificación de los materiales en las categorías precedentes (Tabla 1.1). Estas concentraciones están

referidas a la fracción fina de los sedimentos (diámetro inferior a 63 μm) y expresadas en mg/kg de peso seco. Para dragados inferiores a 20000 m3, los Niveles de Acción 1 se consideran el doble del valor normal.

Tabla 1.1. Contaminantes y NNAA establecidos para la caracterización de dragados portuarios para su vertido al mar.

Compuesto	Nivel de Acción 1	Nivel de Acción 2
As	80	200
Cd	1.0	5.0
Cr	200	1000
Cu	100	400
Hg	0.6	3.0
Ni	100	400
Pb	120	600
Zn	500	3000
Σ_7-PCB	0.03	0.10

Las RRGMD incluyen una primera fase donde se realiza el análisis de granulometría, contenido en materia orgánica y, si existen indicios de posible contaminación en la zona, análisis bacteriológicos (estreptococos, coliformes fecales, etc.). En una segunda fase se realizan los análisis de los compuestos químicos de interés. Se han establecido dos grupos de compuestos. El primero de ellos, de análisis obligado para todas aquellas muestras que requieran caracterización, incluye los metales mercurio (Hg), Cadmio (Cd), Plomo (Pb), Cobre (Cu), Zinc (Zn), Níquel (Ni), Cromo (Cr) y la suma de bifenilos policlorados indicados (congéneres 28, 52, 101, 118, 138, 153 y 180 nomenclatura IUPAC). Si las concentraciones de algunos contaminantes superan el NA1, también se incluye en la caracterización el metaloide Arsénico (As) y otros compuestos orgánicos (PCBs, grasas y aceites, etc.) si ciertos NNAA1 son superados. En general, y en cumplimiento de lo establecido por las Convenciones de Oslo, Londres y Barcelona se han incluido otros muchos compuestos para ser analizados cuando se sospeche que pueden estar presentes en los sedimentos.

Los materiales de dragado pueden ser vertidos al mar libremente o con determinadas condiciones, pueden necesitar medidas de aislamiento o incluso instalaciones cerradas en tierra, o bien pueden ser reutilizados (Fig. 1.1). La opción recomendada, y hacia la cual debe estar dirigida la gestión, es a la búsqueda de usos productivos según las características propias del material: los materiales de dragado entendidos como materia prima y no como

desecho inútil. En caso de no ser posible encontrar usos factibles o bien porque la opción más adecuada sea el vertido al mar, se deben llevar a cabo los estudios necesarios para la localización de la zona de vertido más adecuada. Se considera que los principales efectos van a estar producidos por el aumento de la turbidez en la columna de agua, disminución del oxigeno disuelto y por la deposición en el fondo de los materiales. Mientras que los efectos de la turbidez sólo se consideran significativos si se realizan en zonas especialmente sensibles, los otros dos factores van a producir cambios más significativos principalmente en la comunidad bentónica.

La selección final de la zona de vertido se hará valorando los efectos ambientales así como los costes que pueden suponer las distintas opciones para el promotor de la obra, teniendo en cuenta los usos locales de cada zona de vertido, la caracterización física de los sedimentos y las comunidades del fondo afectadas. En cualquier caso se incluyen algunas medidas para minimizar y/o corregir los posibles efectos biológicos. La Tabla 1.2 resume los estudios necesarios para la solicitud de vertido al mar de dragados portuarios para las distintas categorías de materiales.

Tabla 1.2. Resumen de las categorías establecidas para la gestión de dragados portuarios (CEDEX, 1994).

Categoría	Concentraciones –C-	Posible vertido al mar	Tipo de autorización de vertido	Estudios necesarios para la autorización
I	C< NA1	Vertido libre	Autorización normal	- Estudio sedimentológico y efectos biológicos (causa física/mecánica)
II	NA1< C< NA2	Vertido bajo condiciones controladas	Autorización especial	- Técnica de vertido controlado y justificación. - Hipótesis de impactos - Programa de control ambiental - Medidas correctivas
III	C> NA2	Vertido mediante técnicas adecuadas de gestión		- Estudio de fuentes de emisión para establecer programa de reducción - Descripción de las técnicas de aislamiento y justificación - Hipótesis de impacto - Programa de control ambiental - Medidas correctivas

Nuevo marco de gestión para dragados portuarios

Como se ha descrito anteriormente la calidad de sedimentos y materiales de dragado se ha evaluado tradicionalmente por comparación entre concentraciones de determinados contaminantes medidos en el sedimento y las denominadas guías de calidad de sedimento (en inglés Sediment Quality Guidelines, SQGs). De este modo se pretendía evaluar el riesgo potencial de los contaminantes asociados al sedimento y se han caracterizando los materiales de dragado en España desde que entrasen en vigor las RRGMD en 1994. En la actualidad estas RRGMD están en revisión para ser adaptadas a los nuevos marcos de gestión recomendados por numerosas agencias internacionales. Entre las mejoras se prevén cambios en los contaminantes considerados y para las cuales se han desarrollado NNAA, ya que en las actuales RRGMD se incluyen sólo ocho compuestos metálicos y 7 bifenilos policlorados. Este listado, utilizado aún actualmente, se estableció en los años 70 aunque hoy en día se hayan caracterizado más de 150 los compuestos en sedimentos portuarios no incluidos en este listado (Stronkhorst, 2003). También se ha establecido recientemente la limitada capacidad para predecir efectos biológicos mediante esta aproximación química, principalmente por los cambios en la disponibilidad de los contaminantes de una matriz ambiental a otra y por los efectos aditivos, sinérgicos y antagónicos que pueden producirse a nivel de receptor biológico por exposición a una mezcla de contaminantes y no a un compuesto aislado.

Para evaluar el riesgo biológico de todos los compuestos presentes en su concentración disponible real y teniendo en cuenta todos los posibles efectos sinérgicos, antagónicos y/o aditivos se ha recomendado a nivel internacional el uso de los denominados bioensayos (LC en su Protocolo de 1996; GIPME, 2000; PIANC, 2006). En países como Estados Unidos los bioensayos se han venido utilizando desde hace años para evaluar el riesgo ambiental de sedimentos contaminados y materiales de dragado (US EPA/US ACE, 1998). En otros países como Canadá, Australia o Holanda su uso es más reciente pero ya se han introducido este tipo de metodologías en el marco regulador (Environment Australia, 2002; Stronkhorst, 2003). Asimismo en las RRGMD se incluye la realización de más ensayos, como por ejemplo ensayos de tipo biológico, en caso de que más de tres compuestos de caracterización obligatoria excedan el 75% del límite establecido como NA2 y/o se encuentren elevadas concentraciones de otro tipo de contaminantes que puedan suponer un riesgo para el medio acuático y/o la salud pública. Actualmente se

reconoce la ausencia de metodologías estandarizadas y validadas para tal uso ya que, como para cualquier otra metodología analítica utilizada en el proceso de evaluación, los ensayos biológicos necesitan cumplir ciertos requisitos antes de ser introducidos en un contexto regulador debido a las consecuencias ambientales y económicas relacionadas con la gestión de materiales de dragado.

2. Objetivos e hipótesis

La hipótesis de partida es que los estudios integrados que consideran la contaminación, la toxicidad y el estudio de otros procesos de bioacumulación son los más adecuados para evaluar la calidad de sedimentos y, en concreto, materiales de dragado. Así, esta tesis doctoral pretende, como objetivo final, desarrollar una metodología integrada para la caracterización y gestión de materiales de dragado. Si bien los resultados obtenidos podrían ser sometidos a un estudio más en profundidad caso por caso, prevalece la importancia de los resultados en su conjunto. El desarrollo de este objetivo general implica la consecución de los siguientes objetivos concretos:

1. Evaluar la contaminación de materiales de dragado procedentes de puertos Españoles. Mediante la caracterización físico-química de los sedimentos se relacionarán los niveles de contaminantes con su distribución. Además se realizará un estudio de la caracterización de los sedimentos según las RRGMD y se comparará el uso de los NNAA usados para la gestión de dragados en España con otras SQGs desarrolladas recientemente con el mismo fin en otros países.

2. Determinar la toxicidad de materiales de dragado procedentes de puertos Españoles mediante la aplicación de bioensayos de laboratorio. Para ello se diseñará una batería de bioensayos de toxicidad adecuada para dragados portuarios que servirá para evaluar los efectos biológicos producidos por sedimentos dragados. Los ensayos biológicos se realizarán sobre los sedimentos caracterizados previamente con el fin de estudiar posibles relaciones causa-efecto así como su sensibilidad frente a los contaminantes.

3. Determinar la variabilidad interlaboratorio de determinados ensayos biológicos. Para ello se realizarán ejercicios simultáneos sobre muestras idénticas en distintos laboratorios donde se realizan este tipo de ensayos. El proceso de caracterización de los sedimentos será similar al descrito anteriormente aunque

sólo se contempla en esta fase el estudio de ensayos que cumplan los requerimientos mínimos, es decir, aquellos que se encuentran en un estado de desarrollo más avanzado y, además, sean realizados en otros laboratorios. Con estos ejercicios se pretende establecer el grado de aceptación de estas metodologías en los laboratorios y estudiar la homogeneización de los protocolos y los resultados procedentes de laboratorios distintos al que desarrolló la metodología.

4. Caracterizar la bioacumulación potencial de estos contaminantes en organismos bentónicos mediante el uso de ensayos de laboratorio. Este tipo de ensayos parece recomendable para el estudio de posibles efectos bioacumulativos y de biomagnificación a lo largo de la cadena alimenticia.

5. Proponer un método escalonado de caracterización de la calidad ambiental de material de dragado que permita una mejora en la gestión de estos materiales en España. Este método escalonado estará basado en la inclusión de las metodologías estandarizadas tras la consecución de los objetivos 1 á 4.

3. Zonas de estudio y recogida de muestras

Los estudios descritos en esta tesis se realizaron con sedimentos provenientes de distintos puertos localizados a lo largo de la costa española. Los puertos fueron seleccionados de acuerdo a la cantidad y calidad de los sedimentos, es decir, por la necesidad de realizar operaciones de dragado para asegurar su navegabilidad y por el tipo y grado de contaminación de sus sedimentos. De tres a cuatro estaciones de muestreo fueron recogidas en cada uno, todas ellas –excepto la estación control- representativas de posibles actividades de dragado. Los muestreos se realizaron en el periodo comprendido entre Septiembre de 2001 y Junio de 2004. Los puertos y estaciones de muestreo sometidos a estudio fueron (Fig. 1.2):

- Puerto de la Bahía de Cádiz, localizado en el suroeste atlántico. La zona de la Bahía de Cádiz ha sido ampliamente estudiada y su selección obedece a la ausencia de fuentes de contaminación importantes. Se recogieron sedimentos en cuatro estaciones: una en el margen derecho de la dársena pesquera (CA2), dos en zonas interiores con una alta acumulación de sedimentos y, por lo tanto, susceptibles de ser sometidas a procesos de dragados (CA3 y CA4), y una cuarta estación en el lugar de recogida de los anfípodos utilizados en los ensayos de toxicidad (CA1).

Ésta última estación fue seleccionada como control negativo para los ensayos de toxicidad realizados en el laboratorio.

- Puerto de Huelva. Este puerto se localiza en la Ría de Huelva, también en la costa suroeste atlántica. La Ría de Huelva, formada en el estuario de los ríos Tino y Odiel, está caracterizada por una alta contaminación metálica debido a la proximidad de la Faja Pirítica Ibérica. Se recogieron sedimentos en cuatro estaciones de muestreo: una localizada en la dársena pesquera de la parte más interior de la ría (H1), y tres estaciones más distribuidas a lo largo del estuario (H2, H3 y H4).

- Puerto de Bilbao. Este puerto se encuentra en la Ría de Bilbao, en la costa cantábrica. La Ría de Bilbao, formada en el estuario del río Nervión, está caracterizada por una alta contaminación metálica debido a las numerosas industrias del metal que se localizan en su zona interior. En este puerto se recogieron sedimentos en tres estaciones situadas a lo largo del estuario (BI1, BI2 y BI3).

- Puerto de Cartagena. Este puerto mediterráneo se caracteriza, además de por su actividad marítima, por las actividades industriales relacionadas con la actividad minera desarrollada en esta zona durante décadas. En este puerto se consideraron cuatro estaciones de muestro situadas en puntos representativos de zonas de dragado (C1, C2, C3 y C4).

- Puerto de A Coruña. Este puerto, para el que se consideraron tres estaciones de muestro (CO1, CO2 y CO3) se caracteriza por su gran actividad marítimo-pesquera.

- Puerto de Pasajes. Localizado en la costa cantábrica, este puerto se caracteriza por la contaminación de tipo orgánico de sus sedimentos. Se consideraron tres estaciones de muestreo, PA1, PA2 y PA3, localizadas a lo largo de la zona portuaria.

- Puerto de Barcelona. En este puerto mediterráneo, caracterizado por una gran actividad en el trasiego de mercancías y pasajeros, se recogieron sedimentos en cuatro estaciones de muestreo (B1, B2, B3 yB4) localizadas a lo largo de su recinto interior.

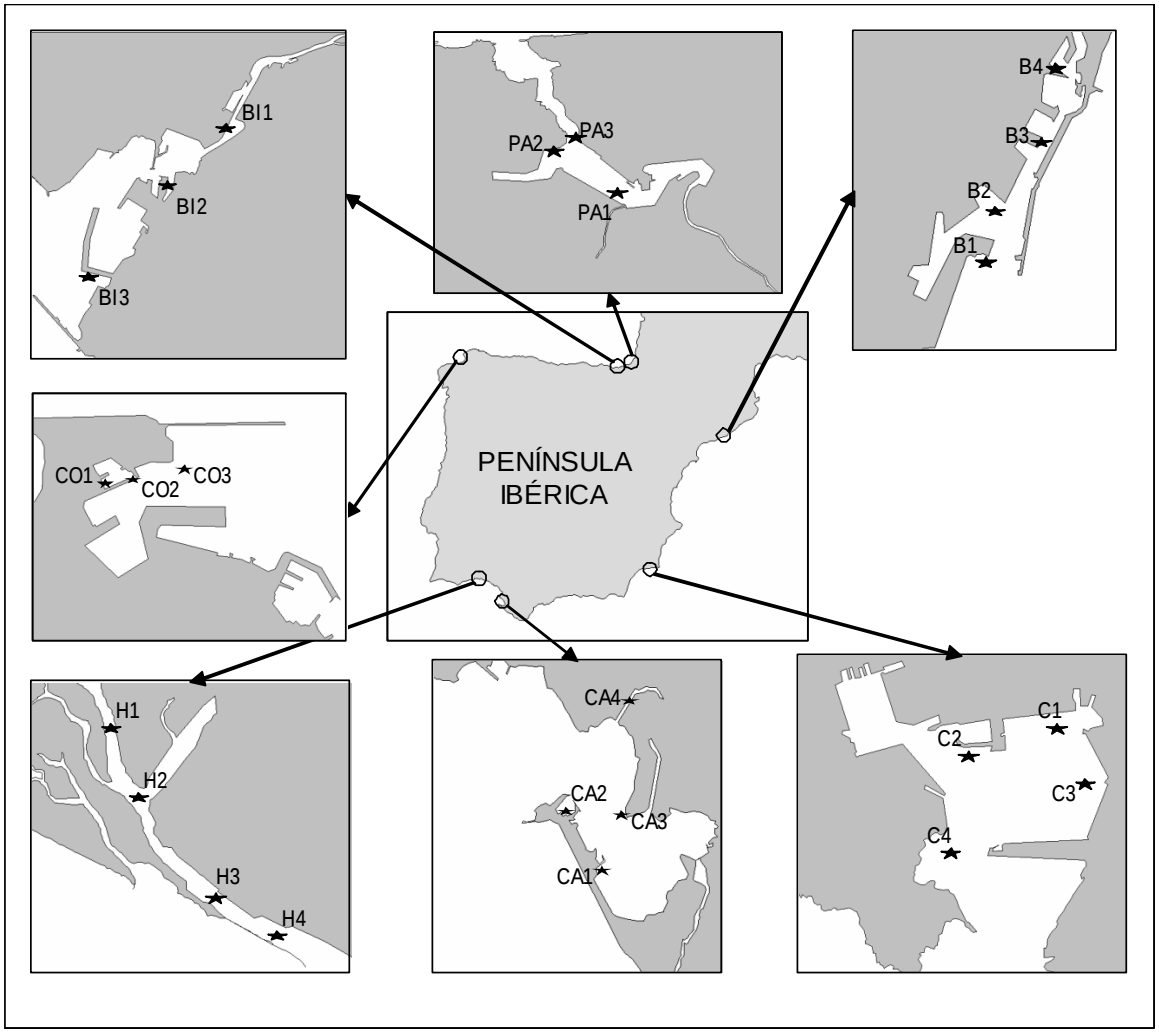

Fig. 1.2. Puertos y estaciones de muestreo seleccionadas en cada zona de estudio.

4. Organización de la tesis

Esta tesis se ha estructurado en siete capítulos, un primero de introducción y descripción de los objetivos de la tesis, cinco capítulos donde se presenta la memoria en sí, y un último capítulo de conclusiones. Cada uno de los cinco capítulos centrales consta de una introducción y descripción resumida en español y los trabajos de investigación escritos en inglés publicados, aceptados, o bien enviados a distintas revistas internacionales. De esta forma en el capítulo 2 se incluyen los trabajos I y II que describen los resultados obtenidos de la caracterización físico-química de los materiales de dragado estudiados.

El capítulo 3 presenta los resultados de la caracterización de la toxicidad mediante ensayos biológicos de estos mismos materiales de dragado. Para facilitar la comparación entre las distintas metodologías se presentan los resultados en cuatro trabajos. En el trabajo III se describen los resultados de los ensayos con el anfípodo *Corophium volutator* y el poliqueto *Arenicola marina,* comparados por ser dos ensayos de diseño muy similar que evalúan los efectos letales tras 10 días de exposición en dos organismos que viven en contacto directo con el sedimento y se alimentan de él. A continuación en el trabajo IV se comparan los resultados de dos especies de anfípodos, una disponible a través de muestreo realizado por el laboratorio que realiza los ensayos y con una manipulación de los organismos mínima, y una segunda no disponible en la zona y que por lo tanto fue muestreada y enviada desde otra zona geográfica española. En el trabajo V se presentan los resultados de los otros ensayos realizados sobre la fase sólida, el ensayo de screening Microtox® y los ensayos con el erizo de mar *Echinocardium ccordatum* y juveniles de almeja *Ruditapes philippinarum*, que evalúan efectos letales tras la exposición durante 14 días, aunque también se ha estudiado el enterramiento como medida subletal. Por último, en el trabajo VI se comparan los resultados de los ensayos realizados sobre los lixiviados de los sedimentos con los resultados de toxicidad por exposición al sedimento en bruto.

El capítulo 4 incluye seis notas de investigación que resumen los ejercicios de intercalibración realizados para estudiar la variabilidad interlaboratorio de éstos. Un primer trabajo (VII) describe el diseño del ejercicio y el estudio de la contaminación de las muestras ensayadas. Los trabajos VIII, IX, X y XI describen los resultados obtenidos para cada uno de los cuatro bioensayos utilizados en el ejercicio interlaboratorio: el ensayo Microtox®, el ensayo con anfípodos, el ensayo con juveniles de almeja y el ensayo con larvas de equinodermo, respectivamente. Finalmente en el trabajo XII se describen las conclusiones obtenidas tras el ejercicio y se realiza un primer análisis multivariante de los

resultados para identificar posibles tendencias en los bioensayos a determinados contaminantes o propiedades del sedimento.

El capítulo 5 consta de tres trabajos donde se estudia el uso de distintos organismos para estudiar la bioacumulación de los contaminantes presentes en los materiales de dragado. Dos trabajos (XIII y XIV) donde se resumen los resultados obtenidos en ensayos de bioacumulación en el laboratorio con poliquetos de la especie *Arenicola marina* para los compuestos de tipo metálico (As, Cd, Cr, Hg, Ni, Pb y Zn) y orgánico (PAHs, PCBs y compuestos orgánicos de Hg), y un tercero (trabajo XV) donde se muestras los principales resultados de los ensayos de bioacumulación de compuestos de tipo metálico con organismos de la especie de almeja *Ruditappes philippinarum*.

En el capítulo 6 se realiza la integración de los resultados mostrados a lo largo de esta memoria. En el trabajo XVII se resumen los principios y las bases para el desarrollo de un método integrado adaptado a una realidad nacional/regional así como los resultados obtenidos durante su optimización para la gestión de dragados portuarios en España. En el trabajo XVIII se propone la integración de las guías de contaminación y ecotoxicológicas para la correcta caracterización del material de dragado y finalmente, en el trabajo XIX se describe la aplicación del método integrado en dos puertos afectados por contaminación metálica y los principales resultados de la aplicación de esta metodología integrada.

Finalmente, en el capítulo 7 de esta memoria, se establecen las conclusiones obtenidas tras la consecución de los objetivos propuestos en esta tesis doctoral.

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Capítulo 2.

Caracterización Físico-Química de los Sedimentos

Como se mencionaba en el capítulo anterior, la calidad de un sedimento se ha evaluado tradicionalmente por comparación entre las concentraciones de contaminantes medidas y determinadas guías numéricas de calidad, las denominadas SQGs. De este modo se pretendía evaluar el riesgo potencial de los contaminantes asociados al sedimento. Asimismo, en España se viene utilizando lo que se ha denominado un sistema basado en Niveles de Acción para la gestión de dragados portuarios. Estos NNAA determinan tres categorías de gestión según dos niveles límite de contaminante: uno inferior –NA1- que determina aquellos materiales que pueden ser vertidos al mar por considerarse afectados por un nivel de contaminación insignificante y cuyo vertido no supone un peligro para el medio ambiente y la salud humana, y un segundo valor límite –NA2- que determina los materiales para los que el vertido al mar no está permitido por considerarse su contaminación lo suficientemente elevada como para producir efectos adversos en el medio receptor.

En este segundo capítulo se pretende estudiar la contaminación de materiales de dragado procedentes de puertos Españoles y para ello, en primer lugar, se han realizado los análisis de las características del sedimento y se han cuantificado algunos de los contaminantes más importantes. Entre los compuestos analizados se han incluido los metales cadmio, cromo, cobre, mercurio, níquel, plomo, zinc y el metaloide As, y compuestos de tipo orgánico (bifenilos policlorados -.PCBs-), todos ellos incluidos en las RRGMD y para los que se han desarrollado NNAA. Además, se han cuantificado otros

compuestos orgánicos del tipo hidrocarburos policíclicos aromáticos (PAHs) por considerarse contaminantes de interés según las actividades antropogénicas realizadas en estas zonas. Para identificar los compuestos de mayor importancia en este tipo de materiales y estudiar el grado de contaminación se han comparado los resultados químicos con los NNAA. De este modo se ha identificado la categoría a la que pertenecerían estos materiales y se han estudiado los resultados de esta caracterización en el conjunto de muestras estudiadas.

Hoy día sigue abierto el debate sobre la validez de este tipo de guías numéricas de calidad, especialmente en relación al uso de éstas como criterios estrictos para la toma de decisiones durante el proceso de gestión. El grupo de expertos en polución en el medio marino (GIPME) en su documento de referencia, aceptado por el Protocolo específico para materiales de dragado del Convenio de Londres para la caracterización de la calidad de sedimentos (GIPME, 2000), considera el uso de las SQGs como una herramienta para la caracterización inicial de la contaminación siempre que estas sean defendibles, y por lo tanto, ayuden eficazmente durante la evaluación, control o prevención de la contaminación. Estas recomendaciones incluyen también un listado reducido de compuestos y sus correspondientes SQGs de aplicación en todos los países firmantes, aunque se contempla la necesidad de establecer un listado más apropiado según las necesidades específicas en cada uno de ellos.

En los últimos años, distintos países están revisando las metodologías utilizadas para la gestión de dragados y esto incluye también la revisión de las guías químicas de calidad de sedimento y el modo en que éstas son utilizadas en el marco de la gestión (Babut et al.; 2003). Una revisión exhaustiva de las SQGs utilizadas por distintos países que tienen un sistema de gestión basado en NNAA evidencia una gran heterogeneidad en el número y en los compuestos para los que se han desarrollado, así como una gran dispersión en el método utilizado para su desarrollo (DelValls et al., 2004; Álvarez-Guerra et al., comunicación personal). Entre las metodologías utilizadas para el desarrollo de este tipo de guías se distinguen tres grandes grupos (Tabla 2.1). Un primer método teórico ha desarrollado SQGs a partir de los equilibrios de partición (EqP) de determinados compuestos entre las distintas fases del sedimento. Para los compuestos de tipo no iónico considera la distribución entre carbono orgánico en sedimento y agua intersticial (DiToro et al., 1991) y para los compuestos iónicos entre los sulfuros ácidos volátiles del sedimento y los metales extraídos simultáneamente (AVS/SEM; DiToro et al., 1992).

Tabla 2.1. Resumen de la guías de calidad de sedimento desarrolladas mediante distintos métodos (DelValls et al., 2004).

Método	SQGs	Referencia	+/-
Teóricas	ESG_{OC} (compuestos no iónicos)	USEPA, 2000	- No tienen en cuenta efectos aditivos, sinérgicos/antagónicos - No tiene en cuenta bioacumulación/biomagnificación en la cadena trófica - Sólo desarrolladas para 37 compuestos no iónicos y 5 metales divalentes
	EqP	Ankley et al., 1996	- Falsos positivos y negativos + Tiene en cuenta la biodisponibilidad y por tanto es de aplicación en sedimentos muy distintos + Tiene en cuenta la causalidad + Recomendado por la USEPA + Amplia base de datos disponible
Empíricas	SSLC	Neff et al., 1986	- Los métodos empíricos usados no muestran efectos causales (no identifican efectos de compuestos determinados) - No resuelven el problema de la biodisponibilidad, se basan en concentraciones expresadas en peso seco - Son específicos para el lugar de estudio + Basados en métodos empíricos para los que se dispone de una amplia base de datos disponible + Buena herramienta de predicción, pero existen falso positivos y negativos
	ERL/ERM	Long et al., 1995	
	AET	PTI Environmental Service, 1991	
	Modelo PAH	Swartz et al., 1995	
	PEL/TEL	MacDonald et al., 1996	
De consenso	TEC/MEC/PEC /EEC	Swartz, 1999	+ Desarrolladas como síntesis de otras guías que incluyen causalidad, biodisponibilidad y efectos de mezclas de compuestos. - Desarrolladas para PAHs.

Un segundo método empírico ha desarrollado SQGs mediante la comparación de bases de datos de contaminantes químicos en el sedimento y efectos biológicos determinados mediante ensayos de toxicidad, observaciones en campo, estructura de la comunidad bentónica, y otro tipo de estudios. Una última aproximación fue introducida

por Swartz (1999) para generar las denominadas guías de consenso. Esta aproximación combina las guías desarrolladas teórica y empíricamente para la elaboración de nuevas guías máximas sin efectos, para medianos efectos, y concentraciones máximas para las que los efectos son probables y extremos.

Parece destacable que la nueva Directiva Marco de Aguas sólo contempla los sedimentos de forma explícita tres veces a lo largo de su texto y siempre en relación al desarrollo de SQGs para proteger la salud humana y la del medio acuático. Estas guías deben ser desarrolladas para los contaminantes de interés, entendidos como aquellos que tienen tendencia a acumularse en los sedimentos según el estudio de partición agua-sedimento, y siguiendo las recomendaciones técnicas establecidas (Technical Guidance Document). Estas recomendaciones revisan de forma exhaustiva la metodología a seguir para el desarrollo de SQGs e intentan armonizar los métodos a nivel europeo siguiendo la tendencia estadounidense del EqP. Recientemente se ha publicado una propuesta para el desarrollo de estas SQG dentro de la WFD (Fraunhofer Institute, 2002), aunque también han sido publicadas ya las primeras críticas a esta propuesta (Crane, 2003).

Los NNAA de uso rutinario en España para la caracterización de materiales de dragado fueron establecidos de acuerdo a los distintos protocolos recomendados a nivel internacional, aunque no han sido validadas como herramienta para la predicción de efectos adversos en el medio marino y, por lo tanto, su fiabilidad parece discutible. Existe una tendencia liderada por países como Francia y Australia para la validación a nivel regional de guías desarrolladas empíricamente en otros países y que parecen ser utilizadas con un cierto éxito a la hora de predecir efectos tóxicos. En el trabajo I este capítulo se presenta la comparación de los NNAA propuestos por el CEDEX (1994) con estas guías de calidad de sedimento, las ERL/ERM desarrolladas por la NOAA (Long et al., 1995).

Entre las mayores limitaciones de estas guías se han establecido un gran número de falsos positivos, con evidencias de un desajuste entre altas concentraciones de contaminantes medidas en los sedimentos y los efectos tóxicos (O'Connor y Paul, 2000), y su uso fuera de las zonas para las que fueron desarrolladas debería validarse. También se han incluido las guías desarrolladas por Riba et al. (2004) basadas en datos de toxicidad de la costa atlántica española, y por lo tanto, de relevancia regional. Entre las metodologías utilizadas para la evaluación de la contaminación se han desarrollado los denominados cocientes medios, que dan un solo valor que integra el exceso de cada compuesto respecto a las SQGs correspondientes, integrando así el número de guías excedidas y la magnitud en que son sobrepasadas (Long et al., 1995; Fairey et al., 2001). Para estos cocientes se han

asignado además unos intervalos asociados a determinadas probabilidades de toxicidad que permiten clasificar los sedimentos en zonas.

De un total de 25 muestras estudiadas sólo 3 de ellas, que corresponden al 12% del total, son clasificadas en la Categoría I según los NNAA, y por lo tanto podrían ser considerados libres de contaminación y ser autorizados para su vertido al mar. Dos de ellas no necesitarían de análisis químicos según el cuadro de gestión por ser sedimentos de granulometría tipo arena/grava. La tercera de las muestras, con un porcentaje de finos y contenido en materia orgánica muy superior, no superó ninguno de los NNAA pero la concentración de hidrocarburos policíclicos aromáticos fue superior al valor límite proporcionado por las guías ERLs. Además, cuando se desarrolla el cociente medio según los ERM, esta muestra también queda clasificada en el grupo de prioridad baja-media, con un valor superior a otras muestras consideradas categoría II según los NNAA.

El 64% de los materiales de dragado eran de categoría III, que incluye materiales no aptos para su vertido al mar y que necesitarían de técnicas de gestión especiales por su alta contaminación. Estos materiales contenían una mezcla de metales y contaminantes orgánicos con concentraciones que variaban en dos o tres órdenes de magnitud, e incluso cuatro en el caso del mercurio. Las concentraciones más elevadas se registraron en los puertos de Cartagena y Huelva, afectados por actividades mineras de importancia, donde el único elemento que no excedió en ningún caso el NA1 ni las SQGs fue el cromo. Los puertos más afectados por contaminación de tipo PCBs fueron los puertos de Pasajes y Cartagena, aunque estos compuestos se encuentran en concentraciones elevadas en todos los puertos excepto en los sedimentos de Huelva y Cádiz. Los compuestos de tipo PAHs, a pesar de no estar incluidos en las RRGMD, registraron concentraciones elevadas en el puerto de Coruña y especialmente en Bilbao. También presentan concentraciones de cierta magnitud algunas zonas del resto de puertos excepto en los de Cádiz y Huelva, donde se encuentran en concentraciones inferiores a los límites de detección.

6 de las 25 muestras estudiadas, que corresponden al 24% del total, se clasificarían dentro de la Categoría II. Para esta categoría de materiales se establece la necesidad de más estudios para evaluar si procede su vertido al mar. Los materiales clasificados en esta categoría contenían una proporción de finos y un contenido en materia orgánica muy variable (entre el 33 y el 99% y el 3 y 24%, respectivamente). Además, estos sedimentos contenían una mezcla de contaminantes de tipo orgánico e inorgánico.

Según la comparación entre NNAA y SQGs desarrollaras de forma empírica, la clasificación de las muestras en cuanto a categorías por excesos es similar independientemente de los valores límites utilizados. Las guías ERL/ERM son más restrictivas que los NNAA y suponen diferencias importantes en la clasificación de algunos materiales por la consideración de los compuestos de tipo orgánico PAHs, cuyas concentraciones son especialmente altas en determinados puertos.

En cuanto al desarrollo de cocientes medios, se pueden aplicar las mismas consideraciones por la introducción de un número mayor de compuestos, aunque este tipo de aproximación parece especialmente interesante ya que ofrece una medida integrada de los números de excesos y el nivel de éstos, y parece una buena aproximación para estudiar la contaminación en términos absolutos de cada muestra o de cada puerto. Utilizando los rangos de toxicidad establecidos por Long et al. (1998) un total de 8 muestras, las cuatro de Cartagena, la muestra 1 de Coruña, 1 y 2 de Huelva y la 4 de Barcelona (éstas últimas todas interiores) se presentan como zonas de máxima prioridad (76% probabilidad de toxicidad). Sólo 2 de ellas, las muestras de mayor tamaño de grano, tendrían una prioridad baja debido al bajo valor del cociente m-ERM-q, mientras que el resto serían consideradas zonas de prioridad intermedia con probabilidades de toxicidad entre 21 y 76%. En cualquier caso, ninguna de las aproximaciones da respuesta a distintos puntos conflictivos en el uso de la caracterización química como única información para la caracterización de los sedimentos, como son en general la débil relación entre toxicidad y categorías según las SQGs, y en particular la ausencia de toxicidad en sedimentos que superan las SQGs más altas y que no producen efectos biológicos adversos (O'Connor y Paul, 2000). Considerando que estas guías se han desarrollado sólo para el 1% de los compuestos que podrían estar presentes en el sedimento, es evidente que esta metodología presenta limitaciones severas.

Como complemento a esta caracterización de la contaminación de los materiales de dragado, en el trabajo II de este capítulo se presentan los resultados tras aplicar a esta misma base de datos, distintas técnicas multivariantes descritas previamente para estudiar la contaminación de sedimentos (ver revisión en Simeonov, 2003) y más recientemente utilizadas para materiales de dragado (Qu y Kelderman, 2001; Cicero et al., 2000). El objetivo de este trabajo es estudiar la distribución de la contaminación e identificar, si es posible, tendencias en la contaminación de los materiales de dragado procedentes de puertos españoles, o de cada puerto en particular. Se considera la utilización de este tipo de técnicas para la mejora del cuadro de gestión de dragados portuarios. En este trabajo se ha aplicado un análisis de tipo "cluster" como técnica para identificar grupos de muestras y

asociaciones entre variables. Además se ha utilizado el análisis de componentes principales para identificar factores "latentes" que puedan describir posibles fuentes de contaminantes o grupos de contaminantes, y que determinan la distribución de la contaminación o tipos de contaminación en el sedimento.

En nuestro caso, la representación del análisis tipo cluster identificó dos grupos de muestras claramente diferenciados determinados por la alta contaminación metálica en los puertos de Cartagena y Huelva. Del mismo modo este tipo de representación permitió agrupar zonas de similares características físico-químicas e identificar los grupos de contaminantes asociados entre sí y aquellos con una mayor relación con las propiedades del sedimento.

El análisis de componentes identificó dos factores principales, uno relacionado con la contaminación por metales y un segundo factor que caracterizaba la contaminación de tipo orgánico en las muestras. Teniendo en cuenta las importantes heterogeneidades entre las zonas de estudio y el limitado número de estaciones incluidas en cada una de ellas, estos resultados apuntan a este tipo de técnicas como una herramienta útil y sobre todo efectiva respecto al coste para la planificación y diseño de campañas de muestreo y caracterización de dragados portuarios en estos puertos y en otros donde deban realizarse este tipo de actividades.

Aunque este tipo de estudios de caracterización físico-química son imprescindibles para el estudio de la contaminación, la información sobre el origen y la distribución de contaminantes no ofrece ningún resultado relacionado con los efectos biológicos que pueden estar relacionados con este enriquecimiento de contaminantes. En este sentido, es necesario utilizar otro tipo de estudios complementarios a éstos que nos den información sobre los efectos biológicos en el ecosistema y sobre la salud humana.

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Using sediment quality guidelines for dredged material management in commercial ports from Spain

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Abstract

Dredged material contamination was assessed in different commercial ports from Spain: Port of Cádiz and Huelva, South West; Bilbao and Pasajes, North; Cartagena and Barcelona, East; Coruña, North West. Sediment from different locations of these ports was sampled and was characterized following the Spanish recommendations for dredged material management. This characterization included grain size distribution, organic matter content and concentration of the chemical compounds included in the list of pollutants and hazardous substances (As, Cd, Cu, Cr, Hg, Ni, Pb and Zn; PCB congeners IUPAC number 28, 52, 101, 118, 138, 153 and 180; PAHs were also analyzed). The results were compared to the limit values of Spanish Action Levels that define the different categories for assessment and management. A set of empirically derived sediment quality guidelines (SQG) was used to assess the possible toxicity of the dredged materials and to improve the use of the chemical approach to characterize dredged material for its management.

Keywords: Contaminated sediments; Harbor; Sediment quality guidelines

1. Introduction

Although anthropogenic emissions to the aquatic environment have been reduced considerably in the late years as control measures were implemented, harbor sediments are still a sink for many pollutants as a result of poor environmental management in the past, diffuse sources and ship accidental spills (PIANC, 1997). The most important groups of contaminants in dredged materials include metals, PCBs and dioxin-like compounds, PAHs, organochloride pesticides (OCPs), oil, radio-nuclides, rare earth metals and organotin compounds (Stronkhorst, 2003, PIANC, 1999).

In order to maintain navigation in large harbors in Spain sediments are periodically dredged (Guerra, 2004). Dredged material management is regulated since 1994 (RRGMD; CEDEX, 1994), namely, the disposal of contaminated sediments into the sea in order to minimize adverse effects in the aquatic environment. After the first physico-chemical characterization, dredged materials are classified in three categories on the basis of the predictable effects of a chemical concentration on the marine biota by comparing the measured chemical concentrations to single-species Sediment Quality Guidelines (SQGs), named Action Levels (AL). Although toxicity studies are explicitly mentioned in the Spanish recommendations to establish the biological significance of sediment-bound contaminants, these tests are not still included in the current decision-making framework for dredged management. In this context, SQGs are being used as a screening tool to assess the biological significance of sediment-bound contaminants in the absence of direct biological effects data (den Besten et al., 2003; Birch and Taylor, 2002; GIPME, 2000).

It is accepted that without defensible SQGs, it would be difficult to assess the extent of sediment contamination (Jones-Lee and Lee, 2005; McCauley et al., 2000). During the last years, several efforts have been devoted to develop environ-

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mental quality guidelines designed specifically to support contaminated sediments and dredged material management and to implement policies and regulatory strategies. Different technical approaches have been used to develop numerical SQGs. Adams et al. (1992) reviewed the three main approaches used in the United States to estimate biological effects of contaminated sediments based on chemical data alone. The equilibrium partitioning model–EqP–has been developed theoretically to account for the factors that likely influence metal and nonionic organic chemicals bioavailability in bed anaerobic sediments (Ankley et al., 1996; Hansen et al., 1996; DiToro et al., 1991). Other approach, the co-occurrence method, developed SQGs empirically using different statistical methods but always on the basis of the observed associations between large data sets of measured adverse biological effects and the concentration of potentially toxic substances present in the environment (Long et al., 1995; MacDonald, 1993). The third of the approaches, named the Consensus Approach and proposed by Swartz (1999), combined sediment guidelines from correlative and EqP approaches to develop consensus SQGs.

Although false positives and false negatives are expected, the EqP methodology is currently adopted by the U.S. Environmental Protection Agency (USEPA, 1995), even if there is a number of research needs that are being addressed, as this approach is implemented including sediment quality modeling, sediment toxicity identification evaluations (TIEs), studies that address bio-availability, studies that address the relative importance of exposure via sediment ingestion or ingestion of contaminated benthos, studies demonstrations of applicability of any SQGs, field verification, extension of the non-ionic mixture models to non-PAH compounds, and the establishment of toxicological databases (with benthic organisms) for standard toxicity endpoints (McCauley et al., 2000).

The National Oceanic and Atmospheric Administration (NOAA) developed a set of empirical SQGs (Long et al., 1995) that provides two values, effects range low (ERL) and effects range high (ERM), which delineate three concentration ranges for each particular chemical and the corresponding estimation of the potential biological effect. The concentrations below ERL represent a minimal-effects range, which is intended to estimate conditions where biological effects are rarely observed. Concentrations equal to, or greater than ERL, but less than ERM represent a range within which biological effects occur occasionally. Concentrations at or above ERM values represent a probable effect range within which adverse biological effects frequently occur. This set of SQGs has been shown to have some predictive ability although do not account for chemical bioavailability and was not based upon experiments in which causality was determined (Long et al., 1998, 2000). These co-occurrence-based SQGs have been widely applied for contaminated sediment assessment (Jones et al., 2005; Roach, 2005; Pekey et al., 2004; Birch and Taylor, 2002; Wakeman and Themelis, 2001; Bothner et al., 1998; O'Connor et al., 1998) even if the suitability has been further discussed together with the potential implications to the regulated community (Lee and Jones-Lee, 1996; Crane, 2003). Nevertheless, the studies to establish regionally action levels and to evaluate the negative effects of contaminated sediments and dredged materials on the biota are under development around the world (GIPME, 2000).

This paper reports the state of sediment contamination in different Spanish commercial ports. This has been done on the basis of evaluations of the sediment chemistry data compared to the single-species sediment quality guidelines used in Spain for dredged material management (the so called Action Levels). In addition, two sets of empirically derived SQGs have been used to study the probability of observing acute toxicity: the ERL-ERM guidelines developed by Long et al. (1995) and the SQGs developed by Riba et al. (2004) using chemical and ecotoxicological data from sediment quality assessment studies in the Atlantic coast of Spain. Finally, the differences when using these sets of SQGs on the decision-making framework for dredged material management in Spain are discussed.

2. Materials and methods

2.1. Sediment sampling

25 sediment samples were collected at 7 commercial ports along the Spanish coast in November 2001 and April 2003. The selection of the sampling sites in each port was based on the need to examine specific point sources (identified by means of available data) and to cover a broad spatial coverage of the ports and thus allowing a general assessment of sediment quality (DelValls et al., 2003). The port area was virtually divided in segments. Three stations were sampled in the ports of Pasajes (PA#), La Coruña (CO#) and Bilbao (BI#) and four in Cartagena, Barcelona, Huelva and Cádiz (C#, B#, H# and CA#, respectively) (Fig. 1). In each site, sediments were collected with a 0.025-m^2 Van Veen grab from approximately the top 20 cm of the sediment and were brought to the laboratory, homogenized and stored at 4 °C and darkness prior to analysis.

2.2. Sediment characterization

All the analyses for sediment physical and chemical characterization were made according to Spanish recommendations for dredged materials and following the recommended protocols (CEDEX, 1994). The dry weight fraction was determined by weight loss at 105 °C. For the rest of analyses, sediments were dried at 40 °C for 24 h. Grain size distribution followed UNE 103 101 and total organic carbon (TOC) content was estimated by loss of ignition (LOI) at 550 °C and gravimetric determination as recommended for small dredged volumes and applying the following expression to express the results as total organic carbon (CEDEX, 1994):

$$\mathrm{TOC}\left(\mathrm{g\ kg^{-1}}\right) = 0.35\mathrm{LOI}\left(\mathrm{g\ kg^{-1}}\right)$$

Metals were determined in microwave acid-digested samples (HNO_3 and aqua regia in a proportion 1:3) in Teflon

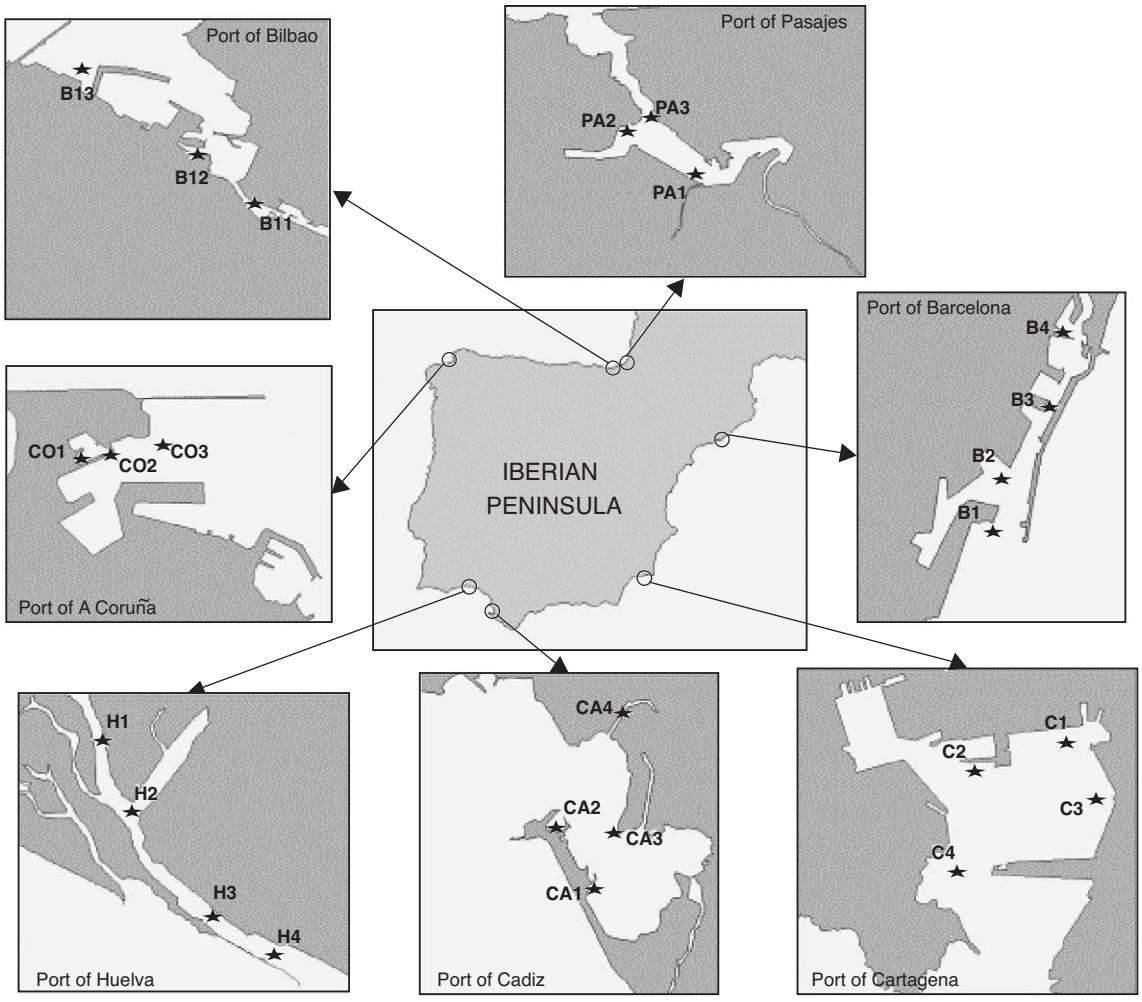

Fig. 1. Map showing the sampling sites of commercial ports. Selected ports are the port of Pasajes (PA#), La Coruña (CO#), Bilbao (BI#) the port of Cartagena (C#), Barcelona (B#), Huelva (H#) and Cádiz (CA#).

vessels and adjusted to volume with boric acid 5.6%. For Hg, the cold vapour technique was used and for As hydride generation, and both quantified using atomic absorption spectrometry. The concentrations of Cd, Pb, Cu, Zn and Cr were determined using flame or furnace atomic absorption spectrometry, depending on the metal content.

PCB congeners 28, 52, 101, 118, 138, 153 and 180 and polycyclic aromatic hydrocarbons (PAHs) were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment and concentration and clean-up with column chromatography. Determination of PCBs was made with gas chromatography with electron capture detection (GC-ECD) (EPA 8080) and 12 PAHs (acenaphtylene, acenaphtene, anthracene, benz(*a*)anthracene, benz(*a*)pyrene, chrysene, dibenz(*a*,*h*)anthracene, phenanthrene, fluoranthene, fluorene, naphthalene and pyrene) were determined with HPLC with fluorescence detection (EPA 8310). Detection limits were 0.8 and 10–30 μg kg^{-1} dry weight of sediment of PCBs and PAHs, respectively. Recoveries of analytes determined ranged from 60% to 120%.

All the analytical procedures were checked with reference materials (Marine Reference Sediment Material for Trace Metal-1, National Research Council (NRC), Certified Reference Material, 277 BCR, and Conseil National de Reserches Canada, 277 BCR, for heavy metals; and NRC-CNRC HS-1 for PCBs and PAHs) and allow agreement with certified values higher than 90%.

2.3. Sediment quality guidelines

To evaluate the sediment contamination and potential ecotoxicological effects associated with the observed concentrations of contaminants, different published Sediment Quality Guidelines (SQGs) have been used (Table 1). In Spain, Action Levels (named AL1 and AL2) are used to characterize dredged material (AL; CEDEX, 1994) and represent hazardous concentrations for organisms based on physicochemical criteria. We used firstly AL1 to identify stations where additional investigations are mandatory (if the AL1 is exceeded for any of the compounds) and AL2 to identify the dredged materials that are not adequate for open water disposal (if any AL2 is exceeded).

Two different sets of empirically derived guidelines were also used to compare the results of the chemical composition. One set is that proposed by Long et al. (1995) although it was derived using data from the U.S Coast: the effects-range-low

Table 1
Sediment quality guidelines for marine sediments included in this study

	CEDEX, 1994		Long et al., 1995		Riba et al., 2004	
	AL1	AL2	ERL	ERM	V1	V2
As	80	200	8.2	70	27.4	213
Cd	1.0	5.0	1.2	9.6	0.51	0.96
Cr	200	1000	81	370	–	–
Cu	100	400	34	270	209	979
Hg	0.6	3.0	0.15	0.71	0.54	1.47
Ni	100	400	20.9	51.6	–	–
Pb	120	600	46.7	218	260	270
Zn	500	3000	150	410	513	1310
Σ_7-PCB	30	100	22.7	180	54	254
Σ_{13}-PAHs	–	–	0.35	2.36	–	–

All values are expressed as $mg \cdot kg^{-1}$ except Σ_7-PCB expressed as $\mu g \cdot kg^{-1}$. AL1 and AL2 are Spanish Action Levels for dredged material management; ERL and ERM are effect range low and effect range medium and V1 and V2 are sediment quality guidelines developed using data from the Atlantic coast of Spain.

(ERL) and effects-range-median (ERM) values. These values represent the concentrations below which adverse effects are expected to occur and are equal to the 10th and 50th percentile concentrations, respectively, of each contaminant represented in the data set that showed significant adverse effects (ERL is the concentration at which adverse biological effects begin to be seen, and ERM is the level associated with adverse effect). Because a small degree of variability that is likely attributable to regional differences in the geochemistry of sediments and the relative bioavailability of sediment-associated toxicants can lead to differences in the predictive abilities of sediment guidelines (Long et al., 2000), a set of SQGs developed using data from the West Atlantic coast of Spain (Riba et al., 2004) has been also used. This set is defined by the highest concentration of a contaminant non-associated with adverse biological effects (V1) and the lowest concentration associated with adverse biological effect (V2). While the ERL and ERM were developed using acute toxicity data, it should be noted that this last set of SQGs was developed using acute toxicity data but also sublethal and histopathological data from laboratory tests.

All these SQGs can be used to assess individual chemicals by comparing the chemical concentration with the limit concentrations or to estimate the probability of acute sediment toxicity and to determine the possible biological effect of combined toxicant groups by calculating mean quotients for a large range of contaminants. This mean ERM quotient (m-ERM-Q) has been calculated according to Long et al. (1998):

$$\mathrm{m-ERM-Q} = \sum(C_i/\mathrm{ERM}_i)/n$$

where C_i is the sediment concentration of compound i, ERM_i is the ERM for compound i and n is the number of compounds. Mean ERM quotients have been related to the probability of toxicity (Long and MacDonald, 1998; Long et al., 2000) based on the analyses of matching chemical and toxicity data from 1068 samples from the USA estuaries. The mean ERM quotient of <0.1 has a 9% probability of being toxic; a mean ERM quotient of 0.11–0.5 has a 21% probability of toxicity; a mean ERM quotient of 0.51–1.5 has a 49% of being toxic; and mean ERM quotient of >1.50 has a 76% of toxicity.

3. Results

3.1. Sediment characterization of conventional parameters

The results of the measured conventional parameters of the samples are included in Table 2. The general characteristics of the sediments vary considerably between ports and between stations: some areas are sandy, whereas others contain a great proportion of fine grain sizes. Most of the dredged sediments from Spanish ports used in this study could be considered fine sediments. Sample CA1 from Cádiz had 99% sand (0.63 μm<size<2 mm) and H4 80% coarse (>2 mm). The percentage of fines (silt and clay, <0.63 μm) for the rest of samples ranged from 31% registered in sample C3 from the port of Cartagena to 99.59% for sample CA4, from Cádiz. Such large variability is also observed for organic matter. Total organic carbon ranged from 1% (samples H4 and CA1) to 24% (sample CA4). The highest value for each port was found for sample CA4, H1, C1, B4, CO2, PA3 and BI3, all values higher than 10% except for CO2. In general, the lowest values were found for the port of Barcelona and Coruña.

3.2. Concentrations of contaminants

Summarized results of the chemical analyses are shown in Table 3. The chemical data indicated that most of the samples

Table 2
Conventional parameters of harbor sediment samples used in this study (Port of Cádiz, CA#; Port of Huelva, H#; Port of Barcelona, B#; Port of Cartagena, C#; Port of Bilbao, BI#; Port of Coruña, CO#; Port of Pasajes, PA#)

Sample	% Coarse	% Sand	% Fines	TOC ($g \cdot kg^{-1}$)
CA1	0.19	99.77	0.04	1.07
CA2	0.05	40.42	59.53	13.75
CA3	0.30	17.80	81.90	20.30
CA4	0.03	0.38	99.59	24.33
H1	0.07	9.71	90.22	20.27
H2	0.19	9.60	90.21	10.64
H3	0.03	56.02	43.95	6.30
H4	80.34	19.65	0.01	1.00
B1	1.43	64.72	33.86	3.06
B2	5.50	57.92	36.58	4.55
B3	3.89	42.13	53.98	4.81
B4	1.41	39.89	58.70	17.56
C1	3.95	38.24	57.81	10.54
C2	5.22	53.59	41.19	9.12
C3	0.93	67.20	31.87	7.19
C4	0.90	50.01	49.10	9.87
BI1	2.39	20.28	77.33	14.81
BI2	38.12	14.48	47.40	15.07
BI3	0.19	6.22	93.59	16.73
CO1	n.a.	n.a.	49.71	5.97
CO2	n.a.	n.a.	84.33	7.53
CO3	n.a.	n.a.	74.75	5.07
PA1	0.84	28.87	70.29	14.43
PA2	3.67	5.08	91.24	18.47
PA3	1.82	38.53	59.65	19.81

n.a. means not available data.

Table 3
Chemical characterization of the dredged materials (Port of Cádiz, CA#; Port of Huelva, H#; Port of Barcelona, B#; Port of Cartagena, C#; Port of Bilbao, BI#; Port of Coruña, CO#; Port of Pasajes, PA#)

	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	PCBs[a]	PAHs[b]
CA1	3.42	0.92	0.10	6.98	0.05	0.06	2.28	21.27	n.d.[c]	n.d.
CA2	30.77	1.32	14.94	202.80	1.98	20.14	86.90	378.25	144.90	n.d.
CA3	16.61	1.23	8.43	46.76	0.28	16.90	17.61	135.50	n.d.	n.d.
CA4	7.81	1.25	14.22	32.07	0.05	21.25	5.14	65.67	n.d.	n.d.
H1	840.00	4.35	32.89	1938.00	2.38	34.57	383.10	2458.00	2.00	n.d.
H2	531.00	2.50	24.10	1497.00	1.99	7.10	384.70	1857.00	2.29	n.d.
H3	273.00	1.32	8.13	772.00	1.20	129.00	217.60	1176.00	n.d.	n.d.
H4	4.70	n.d.	9.70	1.90	0.04	0.80	5.30	20.90	n.d.	n.d.
B1	17.39	0.93	105.20	74.88	0.94	18.87	86.66	253.80	49.20	0.28
B2	21.19	1.52	103.70	159.70	1.12	29.12	103.50	424.00	138.30	0.37
B3	18.56	0.62	59.53	102.10	1.15	22.24	91.90	219.70	85.30	0.61
B4	28.99	2.88	93.86	601.10	4.12	32.30	455.30	1165.00	272.90	1.80
C1	101.50	98.49	66.64	665.90	136.40	29.04	1397.00	8661.00	123.00	0.91
C2	64.71	17.47	45.61	313.40	32.71	15.33	748.30	1885.00	468.20	1.03
C3	88.00	31.88	57.57	453.30	115.20	19.32	1397.00	3310.00	107.60	0.66
C4	62.55	6.79	29.48	171.10	21.59	19.32	486.70	900.80	118.90	1.24
BI1	67.26	2.00	18.27	102.60	0.74	26.39	147.50	476.10	111.60	66.71
BI2	104.00	2.00	23.11	204.10	1.43	32.00	285.90	777.50	256.20	13.90
BI3	21.71	0.04	3.48	23.03	0.18	15.72	40.70	122.35	22.12	0.63
CO1	27.43	0.96	28.67	209.10	6.41	19.90	259.60	513.20	254.40	7.38
CO2	22.50	0.51	31.43	53.12	0.47	19.96	82.37	191.40	58.80	7.07
CO3	13.57	0.25	33.43	35.28	0.54	19.23	54.10	134.90	40.40	1.94
PA1	39.13	0.68	26.73	158.10	1.07	33.49	293.70	1085.00	610.00	n.d.
PA2	28.86	0.70	23.42	167.10	1.29	28.48	246.00	763.00	740.00	1.06
PA3	23.78	0.04	18.61	162.50	1.36	19.61	154.90	576.00	240.00	0.26

Results are expressed as mg kg^{-1} dry weight basis except PCBs, in μg kg^{-1} dry weight.
[a] Σ_7-PCBs.
[b] Σ_{12}-PAHs.
[c] n.d. means not detected.

contained mixtures of contaminants, including trace metals, PAHs, and chlorinated hydrocarbons. The concentration of most trace metals ranged from two to three orders of magnitude and even four for Hg with the lowest values corresponding to the sediments that reported the lowest proportion of fine sediment particles. The highest concentrations for most of the compounds were found in the port of Cartagena and in the port of Huelva: sample C1 showed the highest concentration of Cd, Hg, Pb and Zn and sample H1 of As, Cu and Ni. The concentrations of PCBs were less than the detection limit for most of the samples at the ports of Cádiz and Huelva. The highest concentration was found in the port of Pasajes but these kinds of compounds were also present in the ports of Barcelona, Bilbao and Cartagena. The concentrations of the PAHs were most often less than the detection limits. The highest concentrations were found in the port of Bilbao (samples BI1 and BI2).

3.3. Comparison with SQGs

The comparison with the different SQGs is resumed in Table 4. The two different ways of comparison have been included: the number of single-species limit values exceeded and the mean quotient calculated for the two empirically derived sets of SQGs, using the V2 value reported by Riba et al. (2004) and the ERM value reported by Long et al. (1995). Only three of the samples, 12% of the sediments, did not fail any of the AL1

Table 4
Number of exceeded Action Levels (AL1 and AL2 from CEDEX, 1994) and SQGs (V1 and V2 from Riba et al., 2004 and ERL and ERM from Long et al., 1995) and mean quotients using the ERM and the V2 values

Samples	AL1	AL2	V1	V2	ERL	ERM	m-V2-q	m-ERM-q
CA1	0	0	1	0	0	0	0.15	0.03
CA2	4	1	4	2	7	1	0.61	0.67
CA3	1	0	1	1	4	0	0.25	0.17
CA4	1	0	1	1	2	0	0.21	0.11
H1	6	2	6	6	7	5	2.20	3.15
H2	6	2	6	6	6	5	1.55	2.27
H3	7	2	5	2	7	6	0.85	1.50
H4	0	0	0	0	0	0	0.01	0.02
B1	2	0	2	0	9	1	0.35	0.39
B2	4	1	3	1	7	1	0.55	0.58
B3	3	0	3	0	9	1	0.35	0.44
B4	6	3	7	4	7	5	1.46	1.63
C1	7	6	7	4	8	6	29.83	23.53
C2	6	4	7	5	7	6	6.73	6.11
C3	7	6	7	4	7	6	17.23	18.42
C4	6	3	6	3	7	3	3.60	3.83
BI1	5	2	4	1	9	3	0.62	1.23
BI2	8	2	6	3	9	5	0.92	1.12
BI3	0	0	0	0	2	0	0.09	0.16
CO1	5	2	7	3	6	4	1.15	1.54
CO2	1	0	2	0	6	0	0.24	0.36
CO3	1	0	0	0	5	0	0.17	0.26
PA1	5	1	6	2	7	4	0.87	1.08
PA2	5	1	5	1	7	4	0.90	1.11
PA3	5	1	3	0	6	3	0.46	0.69

values: CA1 in the Port of Cádiz, H4 in the Port of Huelva and BI3 in the port of Bilbao. All the rest of the samples failed any of the AL1 values and a total of 16 samples that account for more than the 50% of the sediments failed at least one AL2 value.

It has been suggested that sediment toxicity is unlikely when bulk concentrations in sediment of all chemicals listed are below the effects-range-low (ERL) value. Conversely, toxicity is probable when any chemical concentration exceeds an effects-range-median (ERM) value (Long et al., 1995). For the set of 25 samples studied, 18 failed at least one of the ERM values, and thus, toxicity can be considered probable for more than the 70% of the dredged sediments. Only two samples, CA1 and H4, did not exceed any ERL value. There are five samples, sample BI3 in the port of Bilbao, two samples in the port of Cádiz, and two samples in the port of Coruña, that showed an intermediate level of contamination and are not included in none of these two categories with different probabilities of toxicity. If we use the SQGs reported by Riba et al. (2004), three of the samples did not exceed any of the V1 values: H4, BI3 and CO3. Those that exceed at least one V2 were in total 16. Six of the sediment samples are not classified in any of both groups.

Mean ERM quotients ranged from 0.02 to 23.53 (values reported for H4 and C1 respectively). Eleven samples showed values higher than 1. When the V2 values are used to calculate the mean quotient, the values ranged from 0.01 to 29.83 (values reported for H4 and C1 respectively). In this case, only eight of the samples reported values higher than 1.

4. Discussion

The first objective of this study is to assess the state of contamination of the selected commercial ports. The ports of Huelva and Cartagena (samples named H# and C#), located in two areas historically affected by mining activities (CEDEX, 1999), reported high concentrations of metallic compounds. The ports of Barcelona, Bilbao, Coruña and Pasajes were mainly affected by Cu, Hg, Pb and Zn and the measured organic compounds at different concentrations. The stations in the port of Cádiz showed a more variable grade of contamination. In this study, one sample showed low levels of contamination (sample CA1), but CA2, located in the inner part of the port, showed higher concentrations of PCBs and Cd, Cu and Hg. For the other two samples from this port, CA3 and CA4, located in the inner part of the bay but not in the inner harbor, intermediate concentrations were registered. This zone has been previously well characterized and has reported low levels of contamination (DelValls et al., 1998a; Campana et al., 2005), but it seems that closer to the inner harbor, unusually higher chemical contaminations are expected in the dredged sediments maybe attributable to the shipping and urban activities. This pattern has also been identified in most of the ports studied since the stations located at the inner part of each port were between the 13 stations that exceeded any of the AL2 values. While this influence is more clear in ports such as Coruña or Bilbao, it is not that clear in others such as Cartagena or Barcelona. In these ports, the contamination registered at the stations is more heterogeneous and there is not a clear contamination gradient. This can be due to particular anthropogenic inputs or as a result from the nature of the particles.

The two samples characterized by the low proportion of fine sediment particles and the lowest organic matter content (CA1 in the port of Cádiz and H4 in the port of Huelva) reported the lowest contamination levels. Nevertheless, the consideration that dredged material contamination is likely to appear together with a high organic matter content and a high proportion of fine grain size (mainly related to urban and industrial wastes) is accomplished in the ports included in this study. The sediment organic content has been shown to be strongly linked to the proportion of fines in the sediment and fine sediments are usually considered to adsorb organic and metallic pollutants more than coarse fractions (Carpentier et al., 2002). In this sense, samples H1 and H2 reported the highest concentrations in the port of Huelva, C1 reported the highest contamination in the port of Cartagena and B4 for the port of Barcelona. Even if fine sediments are highly correlated to organic matter content, correlation analysis on our results did not showed significant associations between the chemical concentrations and these two sediment parameters (data not showed) maybe due to the high variability between the ports.

The characterization process for the dredged sediments tried to mimic as much as possible the characterization process that is usually recommended for dredged material management in Spain but due to the large number of ports, the number of sampling sites for each port have been reduced. The decision-making framework is tiered and proceeds through sequential steps (named tiers) defined as different grades and depths of information. The first tier includes the review of the available information including physical, chemical and biological data that can be reported from the zones of study. While sometimes this information is sufficient to match a dredged material as suitable for open water disposal if no effects are expected, in other cases, further assessments are required, and then the process follows to the next tier. Nevertheless, all the previous information that is reported in this first tier can give clear information (historical sources of contamination, current regimes of the zone, etc.) that can point out possible sinks for pollutants and zones of special concern, such as those in the inner harbor, or can identify zones that are not needed of further chemical characterization because the materials are sandy or coarse sediments. The next tier in the management framework includes the list of contaminants that are analyzed on the sediment fraction <63 μm and is the set of compounds here reported. Undoubtedly, some samples contained chemicals that were not quantified or for which there are no SQGs and then the potential effects cannot be predicted. In this sense, the use of the SQGs make the major assumption that chemical analytes used are indeed representative of the toxicologically significant chemical mixture in the samples regardless of which chemicals were quantified in the analyses. As pointed

by Fairey et al. (2001), this is a simplistic approach because of the infinite number of chemicals in field-collected sediments. The selection of the list of priority pollutants should be site-specific and made according to the particular objectives using the information provided in the first tier, although the use of chemicals that occur most commonly will improve the applicability to a wide range of environmental conditions. In this sense, the use in this study of the list of contaminants developed by the CEDEX (1994) seems justified since it is the one recommended by the Spanish related agencies on dredged material management but this limitation should be taken into account when considering the potential toxicity of the sediments according to the chemical results. Furthermore, the presence of ammonia, hydrogen sulfide or low-dissolved oxygen in dredged materials and contaminated sediments have been reported as the most common causes of sediment toxicity (Lee and Jones-Lee, 1996) but none have been included in the recommendations for dredged material management and the SQGs used in this study.

A résumé of the classification of the samples according to the decision-making framework proposed in Spain for dredged material management is included in Table 5. As mentioned before, the bay of Cádiz has reported low grades of contamination and main sources are related to urban wastes (DelValls et al., 1998b; Lara-Martín et al., 2005). The dredged materials from the inner harbor have shown in this study potential biological adverse effects, but on the other hand, there is a station suitable for beneficial uses or open water disposal that correspond to sandy sediments. The intermediate grade of contamination of the other two samples together with the high percentage of fines and high organic content point out that further assessments are required before the best management option is selected. Other ports such as Huelva or Cartagena stand particular historical sources of metallic pollution, and thus, the dredged sediments reported extremely high concentrations of some compounds that make them not suitable for open water disposal. The rest of ports are between those that stand higher maritime traffic in Spain, although no other important sources of contamination are present. The mixture of compounds and the high concentrations reported, mainly attributed to the port activities themselves, makes the dredged sediments not suitable for open water disposal or is needed of further assessments to ensure that no adverse effect is expected. The set of limit values routinely used to manage dredged sediments in Spain does not classify materials definitely; in most of the studied ports, further assessment is needed to clearly identify the potential toxicity of some sediments, but no recommendations have been established yet describing suitable tools as those available for the chemical characterization. Only the sediments clearly not toxic and those with very high concentrations of contaminants (12% and 64% of the sediment samples, respectively) are effectively classified, remaining a wide zone of uncertain effects.

When co-occurrence-based SQGs are used the number of sediments that do not exceed the lower limit values are quite similar, although some slight differences are found when using the ERL and the V1: if sample CA1 is not included in this category when using the SQGs developed by Riba et al. (2004) due to the lower V1 for the metal Cd, sample BI3 exceeds the ERL for the PAHs, for which AL and V1 have not been developed. Nevertheless, special attention should be paid on sample CA1 since the V1 and V2 values were developed using data from studies on Cadiz and Huelva. This same tendency is found when considering the higher limit values: despite the fact that a total of 64% of sediments are not suitable for open water disposal according to the Spanish Action Levels for dredged materials and this percentage increases to 72% when using the ERMs and to 64% when the V2 values are used, the ports of Cadiz and Huelva report higher number of SQGs exceeded possibly due to the regional specificity of these values.

Both the empirically derived SQGs do not elucidate the potential adverse biological effects of these stations and the number of materials matched in this category is still high (Tables 4 and 6). The m-ERM-q, used to obtain some information about the number of exceeded values and the extent to which the SQGs are exceeded, has been related to four different categories with the related biological adverse effects expected instead of the three included in the Spanish recommendations for dredged material management. Only the two samples H4 and CA1 are classified as “Low-Priority

Table 5
Classification of the different stations and its management requirements according to the Spanish recommendations for dredged material (CEDEX, 1994)

Port	Sample	Potential effects	Management requirements
Cádiz	CA1	Physical	Materials can be freely dumped, normal discharge authorization
	CA2	Biological adverse effects	Isolation and/or bioremediation
	CA3, CA4	Further assessment	Special authorization including biological studies
Huelva	H1, H2, H3	Biological adverse effects	Isolation and/or bioremediation
	H4	Physical	Materials can be freely dumped, normal discharge authorization
Barcelona	B1, B3	Further assessment	Special authorization including biological studies
	B2, B4	Biological adverse effects	Isolation and/or bioremediation
Cartagena	C1, C2, C3, C4	Biological adverse effects	Isolation and/or bioremediation
Bilbao	BI1, BI2	Biological adverse effects	Isolation and/or bioremediation
	BI3	Physical	Materials can be freely dumped, normal discharge authorization
Coruña	CO1	Biological adverse effects	Isolation and/or bioremediation
	CO2, CO3	Further assessment	Special authorization including biological studies
Pasajes	PA1, PA2, PA3	Biological adverse effects	Isolation and/or bioremediation

Table 6
Classification of probabilities of toxicity for each sample according to the calculated mean ERM quotients

Mean ERM quotient	Probability of toxicity (%)	Priority area	Samples
>1.5	76	Highest	H1, H2 B4 C1, C2, C3, C4 CO1
0.51–1.50	49	Medium–high	CA2 H3 B2 BI1, BI2 PA1, PA2, PA3
0.11–0.5	21	Medium–low	CA3, CA4 B1, B3 BI3 CO2, CO3
<0.1	9	Lowest	CA1 H4

Sites", and BI3 would now be a "Medium–Low-Priority Site." Eight sites that represent 36% of the samples had an ERM quotient higher than 1.5, which classifies them as "High-Priority Sites": the four sites located in the port of Cartagena, two sites in the port of Huelva, and one in Coruña and Barcelona. The rest of the samples are classified as "Medium–High-Priority Sites" or "Medium–Low-Priority Sites." The use of the limit values developed for the Atlantic coast of Spain makes some differences when the mean quotient is calculated using the V2. Mean quotients are lower when using the V2 in the ports of Pasajes, Coruña Bilbao or Barcelona, but this can be explained by the absence of limit values for PAHs that are of special concern in these ports. Moreover, the spatial scale at what the different sets of limit values can be used is uncertain and one of the outstanding questions related to the used of SQGs. The cost in time and materials needed to satisfy the minimum data requirements for determining no effects levels for sediment biota is high, and the cost-effectiveness for the different jurisdictions to develop separate SQGs has not been decided. Nevertheless, the confidence of transferring the limit concentrations developed in different jurisdictions is unknown.

Because of the uncertainties related to the SQGs, these are typically conservative; it means over-protective, and only for those samples that have negligible ecological risk, highest reliance and reliability are placed. The number of samples from this study that have been matched in this category is little; thus, little number of false negatives is expected as well (defined as toxic materials that have been incorrectly classified) but we have to consider that according to the Spanish recommendations for dredged material management, two of these three sediments would not need chemical characterization after a first assessment of some conventional parameters of the sediment such as the grain size distribution. One of the approaches to clarify the interpretation of the chemical data in a regulatory context is the use of background concentrations that can inform about contaminant concentrations prior to anthropogenic inputs, but as for the SQGs, the spatial scale at what these values can be used is uncertain. This approach, together with the assessment of the potential risk based on contaminant concentrations at reference areas, used as benchmarks against which to compare the exposed sites have been recommended for dredged material management. These areas, intended to represent the optimal range of minimally impaired conditions that can be achieved at sites anticipated to be ecologically similar, are not easily found, and moreover, they must be acceptable by local stakeholders, reasonable and appropriately represent reference conditions (Krantzberg et al., 2000). Some of the outstanding questions on the development and use of SQGs for sediment and dredged materials have been pointed out in the last years (Crane, 2003; DelValls et al., 2004) with the aim to improve the different decision-making frameworks and to truly evaluate the use of these limit values. Some of the questions, such as the possible weakness of the approach for a mandatory standard or the uncertainties when using the SQGs as mandatory and legally enforceable pass/fail limits, are solved using the SQGs as an early, conservative screening tool in a tiered risk assessment framework. Other questions have been addressed by the related national agencies and the research needed is been carried out, but there are still no SQGs or background levels developed for the regional characteristics that can be applied with confidence.

Although the classification of the dredged materials has been made using different approaches for the development of the used SQGs, the results do not differ that much: commercial ports are zones of concern themselves due to different anthropogenic inputs, and moreover, potential biological effects are likely to occur due to the high concentrations of a mixture of compounds that are expected. Even if the percentages of sediments can vary depending on the SQGs used, the lack of local sediment effect data makes not possible to verify the validity of using the different sets of SQGs. These guidelines are useful as a screening tool to prioritize contaminants or even areas of concern using the medium quotients. Nevertheless, since the list of contaminants in the national recommendations does not include all the chemicals of concern and with possible adverse effects, and moreover, because only in case of extreme contamination the chemical data alone compared to the SQGs are able to predict toxicity, it seems highly recommendable to include toxicity bioassays in the next tiers when managing dredged sediments in Spain as it has been done in other countries (den Besten et al., 2003).

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A multivariate assessment of sediment contamination in dredged materials from Spanish Ports.[1]

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Abstract

This paper summarises the performance of multivariate exploratory analyses to investigate on their potential application for dredged material characterisation and management in Spanish ports. The data from 25 sampling stations located at 7 different Spanish ports, produced a matrix consisting of 300 observations on 10 variables: the metals Cd, Cu, Cr, Hg, Ni, Pb and Zn, the metalloid As, the sum of PAHs and PCB congeners, and the proportion of fines and organic matter content. The cluster analysis was suitable to separate the sediments according to the extent of contamination while the PCA indicated that the data was defined by two principal factors: a first one that accounts for 56.4% of the total variance, describing the metallic contaminants except Ni and Cr, and a second one that accounts for 14.4% of the total variance describing the organic contaminants PAHs and PCBs. The use of these two simple multivariate techniques resulted a cost-effective approach for dredged material characterisation and management with little additional effort.

Keywords: discrimination, cluster analysis, PCA, harbour, dredged material.

1. Introduction

In the last 40 years national governments have become active in their attempts to monitor and control environmental pollution (den Besten et al., 2003) at the time different international conventions on marine environmental protection were implemented. In this context dredged material management has become a key issue for the protection of aquatic ecosystems due to the potential harmfulness during dredging and subsequent relocation of sediments in estuaries and marine ecosystems. Two main factors contribute to pollutant

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accumulation in harbour sediments: 1) ports and waterways are usually an important focus of industrial (shipping, loading and unloading, accidental spills) and urban (waste water emissions) activities and 2) they have been designed to minimise hydrodynamic energy on the inside (Guevara-Riba et al., 2003). The disposal of dredged material is controlled by a license system, which actually requires the issue of a permit previous analysis and scientific evaluation of the dredged material and the intended disposal site (LC, 1996). Economic, engineering, environmental, regulatory and social aspects all need close consideration for dredged material management though information on the physical, chemical and biological characteristics of the sediment are usually necessary to determine potential dredging methods through a tiered approach, focusing step by step more on details when necessary (PIANC, 2006).

As per recommendation of the 1996 Protocol to the London Convention, sufficient information for chemical characterization may be available from existing sources thus a first important step when following this type of assessment framework is to examine all ready available data which can be of some help for dredging operations. In some cases additional measurements may not be required of the potential impact of similar material at similar sites (IMO, 1998) if gathered information is sufficient to make sound conclusions. Nonetheless large-scale studies, designed to monitor sediment contamination in coastal areas that make it possible to compare contaminant levels over different scales of distance and time periods, are available only for few countries. This type of information is scarce only in other countries such as Spain, where environmental quality studies have been performed in some coastal areas, and efficient networks to coordinate at national level in sediment contamination are recent (Viguri et al., 2003).

This paper focuses on the use of untutored multivariate statistical techniques to explore the physico-chemical properties of dredged materials from several Spanish ports. These type of techniques have been used for exploratory data analysis, it is: 1) to determine similarities and dissimilarities between sampling stations and sampling areas; 2) to identify data structure features and trends in the behaviour of pollutants (i.e. De Bartolomeo et al., 2004; Tauler et al., 2004; Stanimirova et al., 1999; Emerson et al., 1997), and 3) for recognising contaminant sources as latent factors which could be interpreted as responsible for the chemical content of the environmental samples (i.e. Loska and Wiechula, 2003; Riba et al., 2002; Simeonov et al., 2001; DelValls et al., 1998). More recently it has been used specifically to implement dredged sediment characterization and management (Qu and Kelderman, 2001; Cicero et al., 2000) as these type of techniques have been recognised

by the related regulatory and scientific communities (OSPAR, 1998). We used the cluster analysis plot to describe the association of samples and variables, and the principal component analysis to evaluate the variability associated with each variable and/or groups of variables. The results are further discussed for a better understanding of sediment contamination in a database comprising several Spanish ports and specifically in the context of navigational dredging and dredged material management in Spain.

2. Materials and methods

2.1. Sampling and sample analysis

The data used in this study included sediments from 7 commercial ports located all around the Spanish coast: three ports are located in the Cantabric coast (Pasajes, Bilbao and La Coruña), two are located in the Atlantic (Huelva and Cádiz) and two in the Mediterranean (Cartagena and Barcelona). Three to four sampling sites were selected in each port trying to cover a wide range of chemical and non-chemical sediment properties and to provide a broad spatial coverage. In each sampling site sediments were collected with a 0.025 m^2 Van Veen grab from approximately the top 20 cm of the sediment and were brought to the laboratory and stored at 4ºC and darkness prior to analysis. The following parameters were determined: As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, sum of polynuclear aromatic hydrocarbons (PAHs) and polychlorinated byphenils (PCBs congeners #28, 52, 101, 118, 138, 153 and 180). Total organic matter content and sediment grain size were included in the analyses as tracers of the solid sediment phase.

Further details concerning sampling strategies, quality assurance and analytical methods have been reported previously (Casado-Martínez et al., 2006). Briefly, the metals were determined in microwave acid-digested samples in Teflon vessels. For Hg the cold vapour technique was used and for As hydride generation, and both quantified using atomic absorption spectrometry. The concentrations of Cd, Pb, Cu, Zn and Cr were determined using flame or furnace atomic absorption spectrometry, depending on the metal content. PCB congeners #28, 52, 101, 118, 138, 153 and 180 and polycyclic aromatic hydrocarbons (PAHs) were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment and concentration and clean-up with column chromatography. Determination of PCBs was made with gas chromatography with electron capture detection (GC-ECD) (EPA 8080) and PAHs (acenaphtylene, acenaphtene,

anthracene, benz(a)anthracene, benz(a)pyrene, chrysene, dibenz(a,h)anthracene, phenanthrene, fluoranthene, fluorene, naphthalene and pyrene) were determined with HPLC with fluorescence detection (EPA 8310). Detection limits were 0.8 and 10-30 μg kg-1 dry weight of sediment of PCBs and PAHs respectively. Recoveries of analyses were in the range of acceptability.

2.2. Environmetric approaches for exploratory data analysis

The data from the 25 sampling stations produced a matrix consisting of 300 observations on 10 variables. This is summarized by presentation of the mean and range of concentrations at each port (Table 1). Values below the detection limit and non detected values were set to one half of the detection limit value. Different approaches were used to pre-treat the data. Specifically the log transformation of experimental data followed by the addition to all variable entries of a constant to remove large negative values was tested. This approach, which is recommended for skewed data sets such as those in environmental studies where the majority of the values are low values with a minor contribution of high values (i.e. Tauler et al., 2004), did not offer any improvement on the results (data not shown). Thus data were just log-transformed before performing the multivariate statistical analyses.

The statistical analyses included firstly a cluster analysis to investigate for similarities between the objects in the variable space, which were represented visually as dendograms (tree diagram) of sampling stations or variables using the Euclidean distance as distance measure and the Ward's method as linkage method. This methodology was selected since it seems a reasonable compromise for quantitative data (Einax and Soldt, 1999; Simeonov, 2001).

In addition, the principal component analysis (PCA) was used as a chemometrical approach to reduce the size of the variable space and substitute a large number of parameters by a small number of independent factors (principal components), which allows data interpretation and data structure explanation (DelValls et al., 1998; Casado-Martínez et al., 2006b). This was done by means of the principal variable loading and the bi-plot of factor scores for the sampling sites in an attempt to correlate both types of information. All statistical analyses were performed with the STATISTICA® 6.0.

3. Results and Discussion

3.1. Sediment characterization

The general characteristics of the sediments varied significantly among and between ports (Table 1). Sediments were principally mud except one station from Cádiz and one station at Huelva. The muddy sediments had percentages of fines between 31% and 99.59% while total organic matter followed a similar trend, varying between 1% for coarse and sandy sediments to 24%, value reported in Cádiz. The chemical characterization showed that most of the sediments contained mixtures of contaminants, including trace elements, PAHs, and PCBs. The highest concentrations for most of the compounds were found in Cartagena and Huelva. The highest concentration of PCBs was found in the port of Cartagena although these type of compounds were also present in Barcelona, Bilbao and Pasajes. In Cádiz and Huelva PCBs were always below detection limits. The PAHs concentrations were most often less than the detection limit with the highest concentrations present in the port of Bilbao.

Table 1. Range of physico-chemical characteristics in sediments from Spanish ports (all concentrations in mg/kg dry weight except PCBs in µg/kg dry weight). N.a. means not available data; n.d. means not detected (see section 2.1).

Port	% coarse	% sand	% fines	TOC (g kg^{-1})	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	ΣPCBs[a]	ΣPAHs[b]
Cádiz	0.03-0.30 (0.14)	0.38-99.8 (39.6)	0.04-99.6 (60.3)	1.1-24.3 (14.9)	3.4-30.7 (14.7)	0.92-1.3 (1.2)	0.1-14.9 (9.4)	7.0-202.8 (72.2)	0.05-2.0 (0.59)	0.06-21.3 (14.6)	2.3-86.9 (28.0)	21.27-378.3 (150.2)	n.d.-144.9	n.d.
Huelva	0.03-80.4 (20.2)	9.6-56.0 (23.8)	0.01-90.2 (56.1)	1.0-20.3 (9.6)	4.7-840 (412)	n.d.-4.4 (2.8)	8.1-32.9 (18.7)	1.9-1938 (1052)	0.04-2.4 (1.4)	0.8-129 (42.9)	5.3-384.7 (247.7)	20.9-2458 (1378)	n.d.-2 (2.2)	n.d.
Barcelona	1.4-5.5 (3.1)	39.9-64.7 (51.2)	33.9-58.7 (45.8)	3.1-17.6 (7.5)	17.4-29.0 (21.5)	0.62-2.9 (1.5)	59.5-105.2 (90.6)	74.9-601.1 (234.5)	0.94-4.1 (1.8)	18.9-32.3 (25.63)	86.7-455.3 (184.3)	219.7-1165 (515.6)	49.2-272.9 (136.4)	0.28-1.8
Cartagena	0.9-5.2 (2.8)	38.2-67.2 (52.3)	31.9-57.8 (45.0)	7.2-10.5 (9.2)	62.6-101.5 (79.2)	6.8-98.5 (38.7)	29.5-66.64 (49.8)	171.1-665.9 (400.9)	21.6-136.4 (76.5)	15.3-29.0 (20.8)	486.7-1397 (1007)	900.8-8661 (3689)	107.6-468.2 (204.4)	0.66-1.2
Bilbao	0.19-38.1 (13.6)	6.2-20.3 (13.7)	47.4-93.6 (72.8)	14.8-16.7 (15.6)	21.7-104 (64.3)	0.04-2.0 (1.4)	3.5-23.1 (15.0)	23.0-204.1 (109.9)	0.18-1.4 (0.78)	15.7-32.0 (24.7)	40.7-285.9 (158.0)	122.4-777.5 (458.7)	22.1-256.2 (130.0)	0.6-66.7 (27.1)
Pasajes	0.84-3.7 (2.1)	5.1-38.5 (24.2)	59.6-91.2 (73.8)	14.4-19.8 (17.6)	23.8-39.7 (30.6)	0.04-0.70 (0.47)	18.6-26.7 (22.9)	162.5-167.1 (162.6)	1.1-1.4 (1.2)	19.6-167.1 (27.2)	54.9-293.7 (231.5)	576-1085 (808.0)	240-740 (530.0)	n.d.-1.1 (0.66)
A Coruña	n.a.	n.a.	49.7-84.3 (69.6)	5.1-7.5 (6.2)	13.6-27.4 (21.2)	0.25-0.96 (0.58)	28.7-33.4 (31.2)	35.3-209.1 (99.2)	0.47-6.4 (2.5)	19.2-20.0 (19.7)	54.1-259.6 (132.0)	134.9-513.2 (279.8)	40.4-254.4 (117.9)	1.9-7.4 (5.5)

[a] Σ_7-PCBs; [b] Σ_{12}-PAHs.

3.2. Multivariate statistical methods

3.2.1. Cluster analysis

The result of the cluster analysis performed on the sampling stations (Fig. 1) presents the formation of two major clusters, each one with different subgroups. The first cluster includes the most severe polluted sediments from Cartagena (sites C1 and C3, located in the east bay), Huelva (sites H1, H2 and H3) and Bilbao (site BI1), all characterised by high metallic concentrations. A subgroup was formed for sites at Cartagena due to its determining concentrations of Cd, Hg, Pb and Zn, which in turn was separated from the three inner stations at Huelva and the inner sediments from Bilbao. The ports of Huelva and Bilbao are located in the Tinto-Odiel and the Nervión estuaries, respectively and both are affected by surface metal enrichment. In Bilbao it is caused by effluent discharge from metal-working industries and subsequent deposition due to reworked upstream mine-waste (Cearreta et al., 2000) while in Huelva it is due to acid mine drainage (Usero et al., 2001). This hypothesis would also explain that station 1 from Huelva (H1), located in the fishing harbour, was not clustered with the other inner harbour sediments due to its metallic contamination

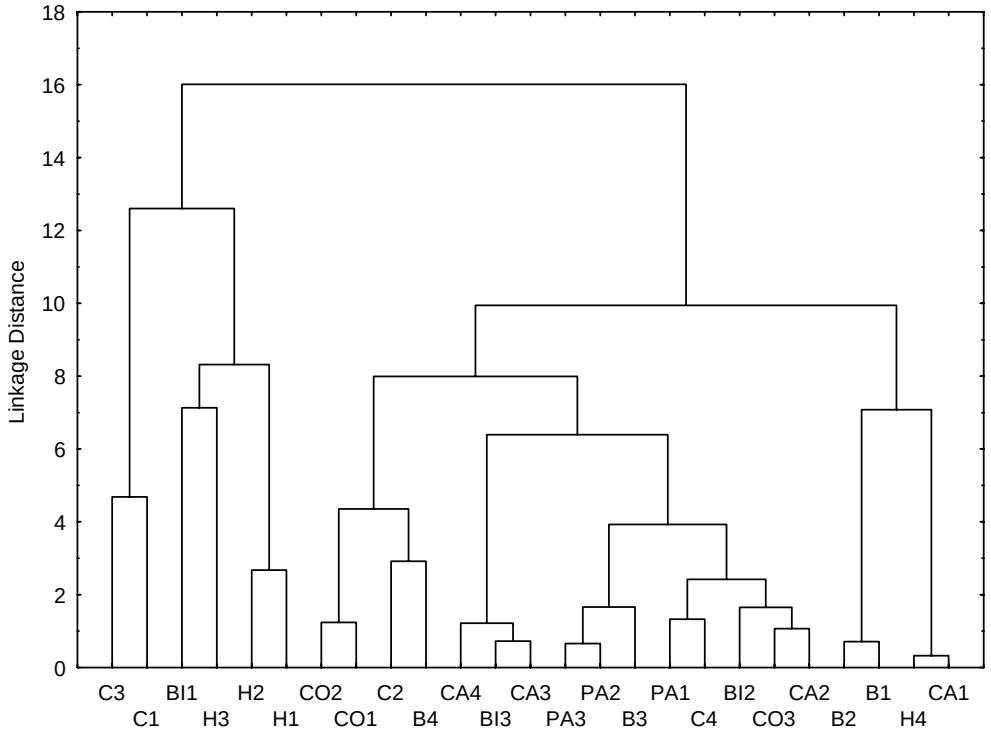

Fig. 1. Hierarchical dendogram for sediment data clustering of sampling sites.

The second cluster comprised the moderately and low contaminated sites, which are clustered in different subgroups according to different sediment properties and characteristics. One cluster was formed by sediments with the lowest proportion of fines and organic matter content. Sites 1 from Cádiz (CA1) and 4 from Huelva (H4) were linked at the lowest linkage distance due to their particular sediment properties and low contamination, which in turn were linked to a new cluster with sites 1 and 2 from Barcelona (B1 and B2), that reported the lowest proportion of fines and organic matter content among the harbour mud. The higher chemical concentrations in Barcelona cause the large linkage distance with the not polluted sediments CA1 and H4.

The other sediments were clustered together, with secondary clusters according to different grades of contamination. A first group represented the high contamination of sites 1 and 2 from A Coruña (CO1 and CO2), station 2 from Cartagena (C2) and 4 from Barcelona (B4), which are more affected by harbour activities due to its location in the inner part of these ports. Site 2 from Cádiz (CA2), site 3 from A Coruña (CO3), 2 from Bilbao (BI2) and, at a higher distance, site 1 from Pasajes (PA1) and 4 from Cartagena (C4) were also affected by harbour activities but less than the sediments mentioned above. In this way, sites 2 and 3 from Pasajes (PA2 and PA3) and site 3 from Barcelona (B3) formed a third group of medium-low contaminated sediments, and finally sites 3 and 4 from Cádiz (CA3 and CA4) and site 3 from Bilbao (BI3) formed a group of mud with low contamination.

The cluster analysis of the variables according to Ward (Fig. 2) identified two major clusters that separate chemical compounds that are linked to the sediment properties (percentage of fines and organic matter content) from those that are not. A first group was formed by the pairs of metals Hg and Pb and Zn and Cd, both at similar linkage distances. The most conspicuous concentrations of these metals were present in Cartagena, that stands important contamination sources: a Zn smelter, that explains the close association between Cd and Zn as these two elements are closely associated in its geochemistry, with Cd being obtained as a by-product from smelting of sulphide ore minerals in which it has substituted for some of the Zn (Alloway, 1995), and Hg and Pb that seem to be more related to other industrial sources of metallic contamination such as a Pb smelter or other metal processing industries. In addition previous studies have shown a close relationship between these four metals in areas heavily affected by steel plant contamination (Romano et al., 2004).

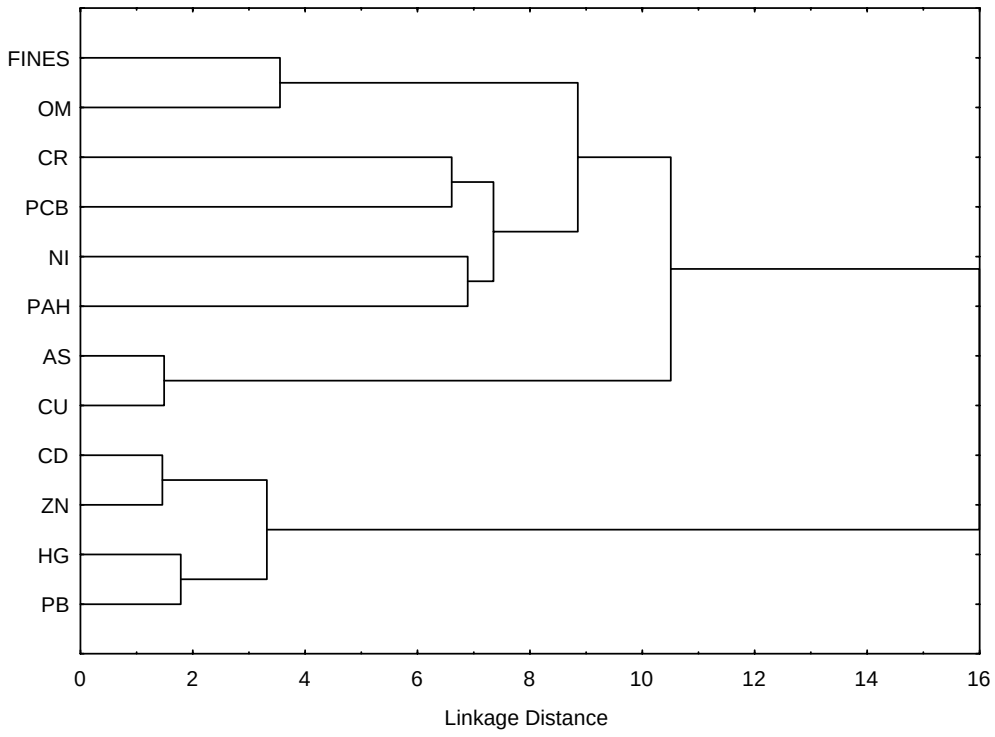

Fig. 2. Hierarchical dendogram for sediment data clustering of variables.

The second cluster comprised other chemical compounds, the proportion of fines and organic matter content. These two sediment properties appeared linked at a low distance indicating that organic matter adsorbs onto fine particulates in harbour sediments, possibly due to the low hydrodynamic energy in the inside. Furthermore, these variables are used as tracers of the solid phase sediment in data interpretation. Organic matter content reveals important complexation processes in the sediment phase with participation of anthropogenic effects and the grain size is related to the processes of metal adsorption (Simeonov et al., 2001; Stanimirova et al., 1999). Previous results have reported the strong complexation of metals by organic ligands, particularly Cu, Ni, Cd and Zn (Förster and Wittmann, 1979; cited by Qu and Kelderman, 2001; Cobelo-García and Prego, 2004), but the cluster analysis evidences a close relationship between the sediment properties and the pairs of compounds formed by Ni and PAHs, Cr and PCBs, and also As and Cu at a higher distance.

The relationship between As and Cu is explained by the high concentrations of these metals in the sediments from Huelva, that show similar patterns in nature due to their common sources and their association with same type of carrier particles with similar sedimentological properties. Similarly, the sediment organic fraction is supposed to play a major role in binding hydrophobic compounds (Culotta et al., 2005), which explains the

cluster of PAHs and PCBs with the organic matter content of the sediment. Nonetheless PCBs were linked to Cr while PAHs were grouped with Ni, which indicates a more complex distribution of these compounds in the sediments studied.

The correlation matrix was developed to study the distribution of variables in pairs (Table 2). As expected the highest correlations were found for the pairs As-Cu, Cd-Zn and Hg-Pb (0.95, 0.96 and 0.93 respectively), even though similar coefficients appeared between the pairs Cd-Hg, Cd-Pb, Zn-Hg and Zn-Pb. On the contrary no significant correlation was found between the other four variables considered, namely PAHs, PCBs, Ni and Cr. Even if the correlation analysis did not identify an straightforward relationship between these compounds, it is worthy mentioning that this high variability may be determined by the more homogeneous distribution of these compounds among the ports studied.

Table 2. Binary correlations (coefficients) of the variables.

Variable	FS	OM	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	PCBs	PAHs
% fines (FS)	1.00											
Organic Matter (OM)	0.74**	1.00										
As	0.30	0.19	1.00									
Cd	-0.09	-0.06	0.03	1.00								
Cr	-0.20	-0.26	-0.07	0.28	1.00							
Cu	0.26	0.20	0.95**	0.19	0.08	1.00						
Hg	-0.18	-0.12	0.00	0.92**	0.29	0.16	1.00					
Ni	0.06	0.04	0.23	0.01	-0.02	0.26	-0.03	1.00				
Pb	-0.12	-0.03	0.17	0.82**	0.33	0.35	0.93**	0.05	1.00			
Zn	0.00	0.03	0.28	0.96**	0.28	0.45*	0.88**	0.11	0.87**	1.00		
PCBs	0.10	0.24	-0.24	0.00	0.09	-0.15	0.01	0.01	0.18	0.05	1.00	
PAHs	0.14	0.10	-0.07	-0.07	-0.13	-0.13	-0.09	0.01	-0.09	-0.09	-0.01	1.00

3.2.2. PCA

Due to the large variability among ports the possibility of extracting seven principal components, each one explaining the variability associated to each area, was considered and seven factors were firstly explored. Nonetheless the results indicated that two to four factors were enough to explain the variance associated to the data studied. The first extracted factor, that explains more than 50% of the total variance, includes all metallic

compounds except Ni and Cr, which were explained in later extracted factors (Table 3). While this first factor seems to explain most of the variability associated to the metallic compounds, the second extracted factor, that explains 14% of the total variance, accounted principally for the organic contamination including both PAHs and PCBs. The third extracted factor, that explained 12% of the total variance, included the sediment properties and Ni while the variability associated with Cr was explained by a fourth extracted that also included Ni, Pb and PCBs.

Table 3. Factor loading and percentage of the total variance explained for 4 factors. Factor loadings >0.60 are marked with an asterisk.

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
% fines	0.24	0.18	0.86*	0.29
Organic matter	0.16	0.10	0.93*	- 0.02
As	0.73*	- 0.36	0.38	0.14
Cd	0.87*	0.13	0.00	- 0.06
Cr	0.30	0.21	0.23	0.84*
Cu	0.79*	- 0.06	0.48	0.25
Hg	0.86*	0.30	0.06	0.30
Ni	0.23	0.11	0.73*	0.55
Pb	0.79*	0.29	0.29	0.41
Zn	0.86*	0.11	0.35	0.29
PCBs	0.21	0.75*	0.14	0.44
PAHs	0.05	0.89*	0.13	0.03
% Explained Variance	56.4	14.4	12.4	5.3

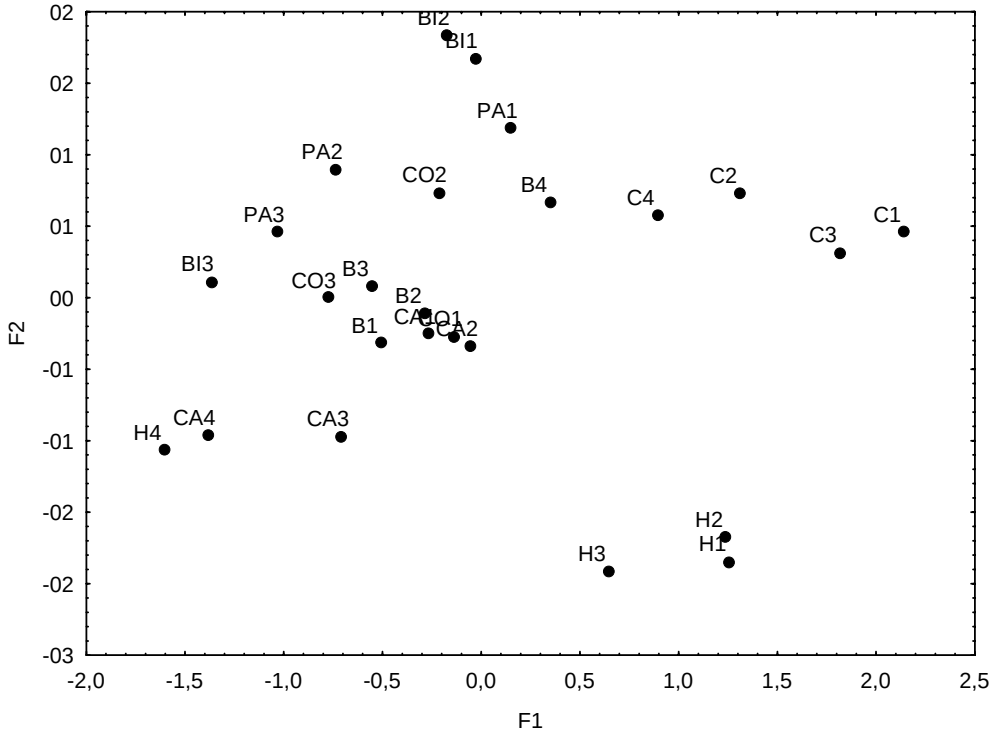

Fig. 3. Principal component score plot of sampling sites from Spanish Ports.

Since the two first extracted factors accounted for a great proportion of the variability associated to metallic and organic contaminants, we explored the distribution of the sediment samples in the space defined by these two factors (Fig.3). A first area of low contamination is represented when F1 and F2 had negative factors. The sediments from Cádiz and sample H4 were located in this area according to the low chemical concentrations, nonetheless some influence of the harbour activities is suspected due to the presence of samples CA2, B1 and B2 from Barcelona, with negative scores for F1 and F2 but a clear increasing trend according to the increasing organic and metallic contamination in the inner harbour sediments (Fig. 4). Sediments from A Coruña (CO#) also presented low factor scores for F1 and F2, although increasing scores for F1 and F3 indicate some surface metal enrichment related to the proportion of fines. Samples CO1 and CO2 reported similar PAHs concentrations while CO1 had a PCBs concentration five-fold than that for CO2, which may explain the need of two factors, F2 and F4, instead of a single one (F2) to explain the organic chemical contamination in this port. In addition high scores for CA3 and CA4 in F3 point out some organic matter enrichment in the inner sediments from Cadiz due to recent urban wastes (DelValls et al., 1998).

The variability for the port of Barcelona appeared explained principally by F4. This factor, that reported decreasing values moving inside the harbour (Fig. 4), was the most

difficult to interpret. Nonetheless, F1 and F2 indicate a clear trend in the organic chemical load of the sediments to increase along the harbours of Barcelona. This was also observed in the port of Bilbao and the port of Pasajes and both showed a clear contamination gradient seaward along the area, with the highest scores for PA1 and a clear decreasing trend for PA2 and PA3. The type of contamination is principally organic, with high positive scores in factors that account for these compounds and some metal enrichment in the inner sediments (Fig. 3). As for other ports, some variability of Pasajes was also explained by F4, which principally accounts for Cr with some other contributions of other compounds such as PCBs, Ni and Pb (Fig. 4).

A clear group was formed by sediment samples from Huelva H1, H2 and H3, with high loadings for F1 and negative loadings F2 (Fig. 3). In the case of Huelva some conclusions are reached after considering the chemicals of concern in the area and the factor loadings for the sediments along the estuary (Fig. 4). Considering that As and Cu reported the highest contamination levels it is of significance the clear decreasing trend in F1 and F3 loadings seaward along the estuary, which is in accordance with previous results reporting a clear sediment contamination gradient along the Tinto and Odiel estuary for some metals (Fernández-Caliani, 1997; Usero et al., 2001) and a clear gradient in the organic content and the proportion of fines. Similarly a group was formed by sediments from Cartagena located up on the right side (Fig. 3), which corresponds to sediments with high metallic and organic contamination. The results gave a very complete distribution of contaminants inside the bay of Cartagena (Fig. 4), with a clear source of metallic compounds on the eastern bay further discussed above that correspond to high scores in factor 1 for sediments C1 and C3 and lower in the sediments from the western bay. On the contrary organic contamination, accounted for in F2, trend to increase in the west of the bay due to the proximity of the principal harbour facilities though a secondary source of PCBs is possible in the vicinity of station C3 according to the high score for F4.

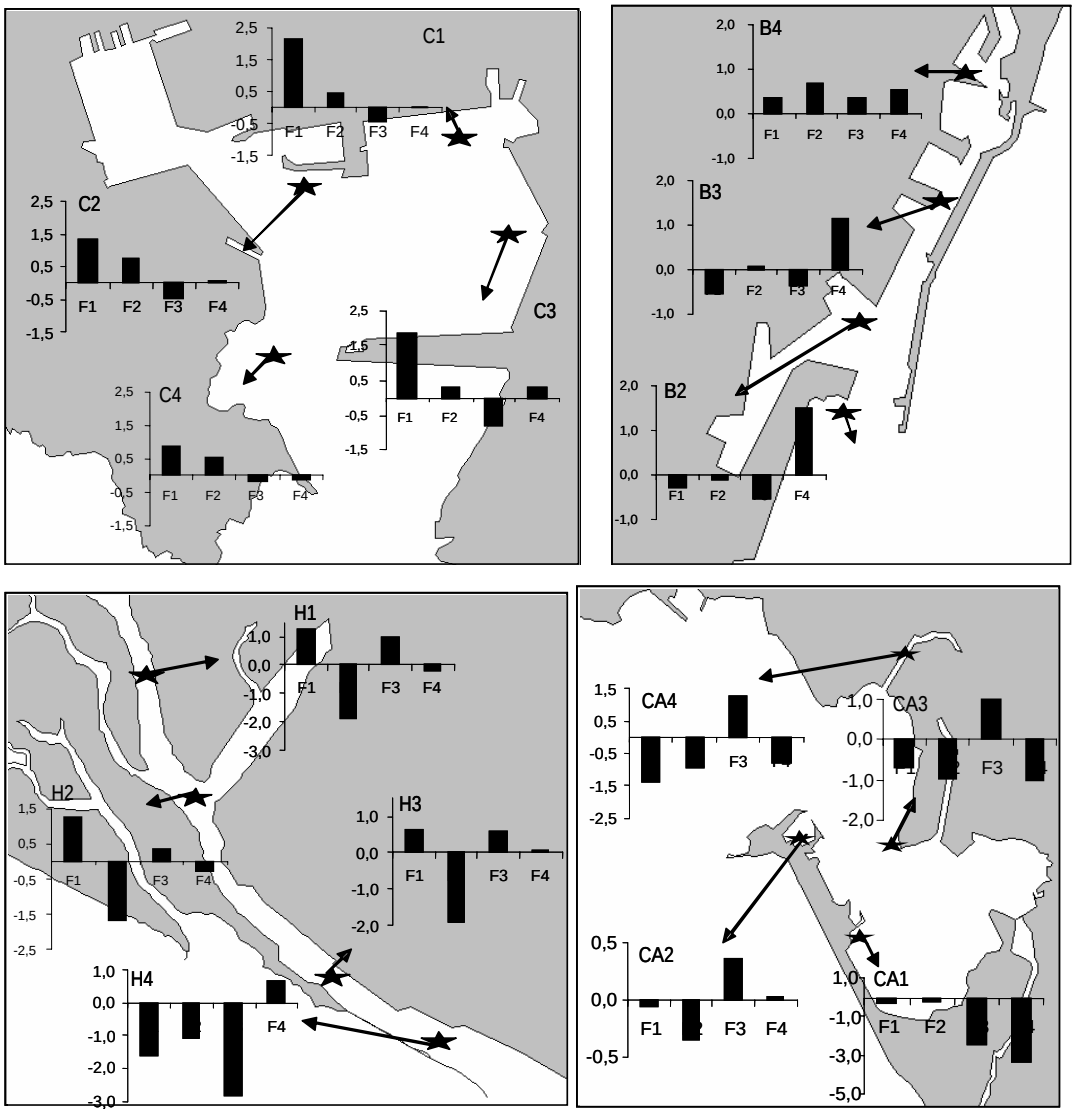

Fig. 4. Sampling stations and loadings for the extracted factors.

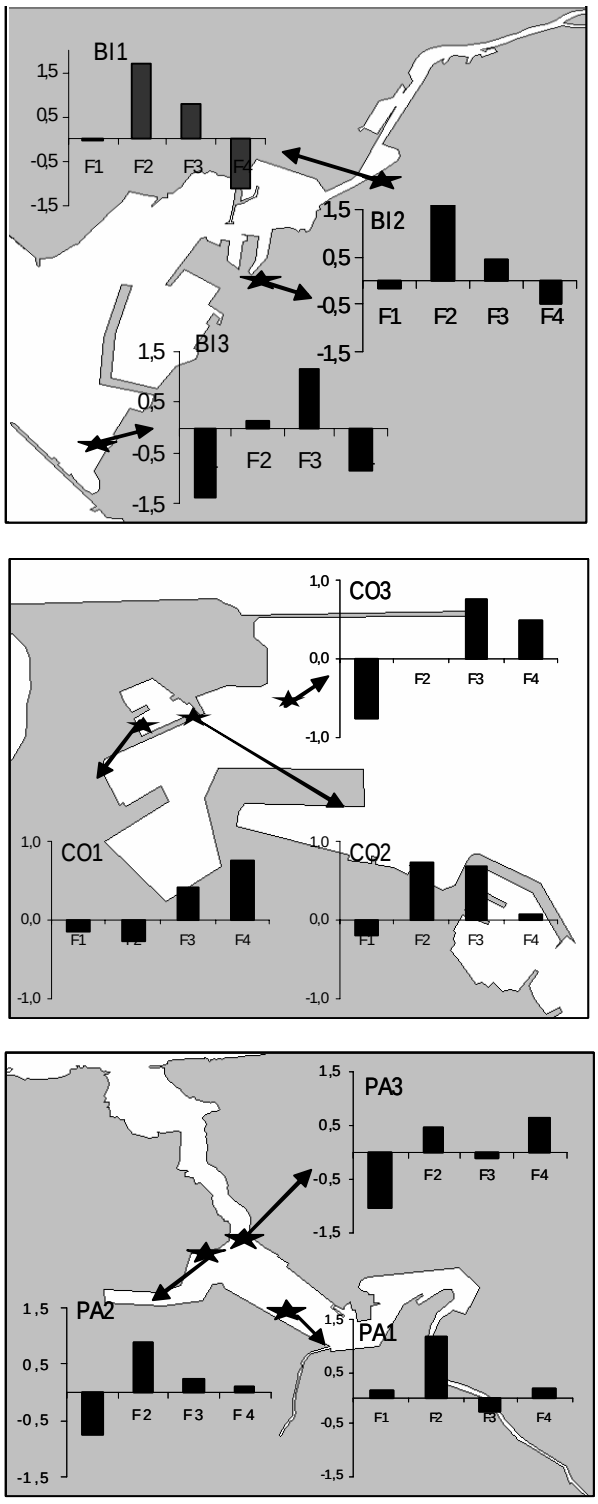

Fig. 4. Sampling stations and factor loadings for the extracted factors. (Cont.)

4. Conclusions

The database available in this study presented a high variability, with very different chemical concentrations and sediment properties among ports and between sampling stations. Nonetheless, these and other examples on the use of multivariate statistical techniques indicate that simple untutored tests may provide a useful tool to study dredged material contamination. Considering that the same statistical software performs several tests with little extra effort, the application of this type of techniques on existing data, or data from pilot or screening studies, can help to estimate distributions in relevant physico-chemical measurements. In this sense, its application to design simple models for a better selection of sampling strategies seems further recommended (MacKnight, 1991). Later on, this type of studies is a known valuable source of information that reduces the costs of the investigations without reducing the confidence on the final assessment (Luoma and Fisher, 1997).

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Capítulo 3.

Caracterización de la toxicidad mediante ensayos de laboratorio

Dentro del cuadro de gestión propuesto en el capítulo 1 de esta tesis se recomienda el uso de ensayos de toxicidad para evaluar la nocividad de los materiales de dragado con concentraciones intermedias de contaminantes, ya que se considera una zona de incertidumbre a la hora de establecer el riesgo ambiental mediante el único uso de las guías químicas. Este cuadro de gestión fue establecido de acuerdo al recomendado por diversas agencias internacionales y grupos de expertos (GIPME, 2000; PIANC, 2006) y ha sido ya introducido en diversos países bajo distintas aproximaciones. Por ejemplo, Holanda ha introducido recientemente el uso de determinados ensayos biológicos, en concreto del ensayo a 10 días con anfípodos de la especie *Corophium volutator*, el ensayo Microtox® SPT y el ensayo DR-CALUX®, de forma conjunta y a un mismo nivel con el análisis de determinados contaminantes de interés. Otros países han incluido este tipo de ensayos pero no han especificado cuales son los ensayos que deben ser introducidos, dando así una mayor flexibilidad a la hora de decidir entre las metodologías disponibles aquellas que se adapten mejor a cada caso en particular. Esto se debe a que existen numerosos bioensayos que han sido desarrollados para la evaluación de la calidad de sedimentos y que se encuentran estandarizados en mayor o menor medida.

En España son numerosos los laboratorios que han desarrollado y tienen establecidos ensayos de toxicidad para la evaluación de la calidad de sedimentos, pero su uso con materiales de dragado es limitado o más bien nulo (DelValls et al., 2001). Como

bien se mencionaba en el capítulo 1 de esta tesis, los costes ambientales y económicos asociados a la toma de decisiones justifican la investigación invertida para el desarrollo y estandarización de cualquier metodología utilizada en un contexto regulador. Son numerosas las agencias competentes en relación a la gestión de dragados portuarios que han colaborado y siguen colaborando de forma activa con la comunidad científica para la continua mejora de las técnicas utilizadas. El protocolo más completo para el desarrollo de bioensayos de toxicidad para ser usados en un contexto regulador fue publicado por Dillon en 1994 (Tabla 3.1) con la finalidad de ofrecer un marco para la evaluación del estado de desarrollo de cualquier bioensayo e identificar las principales deficiencias.

El objetivo de este capítulo no es proponer nuevos bioensayos de toxicidad para la gestión de materiales de dragado, ya que existen numerosos ensayos ya estandarizados internacionalmente para la gestión de materiales de dragado y/o para la evaluación de la calidad ambiental. Haciendo uso de este desarrollo se propone una batería de bioensayos para la caracterización de materiales de dragados en el contexto de las RRGMD utilizadas en España. Con este objetivo, se llevó a cabo una fase previa de planificación del trabajo de laboratorio en la que se realizó una amplia revisión bibliográfica y de consulta a expertos internacionales y nacionales con la finalidad de obtener un inventario de los ensayos de toxicidad disponibles. Así como toda aquella información que pudiese ser considerada de interés dentro del cuadro de desarrollo planteado para seleccionar posteriormente los bioensayos potencialmente más adecuados para su uso en este contexto (Tabla 3.2).

Los ensayos seleccionados se clasificaron en dos grupos distintos según la finalidad con la que fueron diseñados: 1) ensayos diseñados para evaluar efectos biológicos y 2) ensayos diseñados para evaluar la bioacumulación potencial de compuestos asociados a sedimentos. Se distinguieron tres tipos de ensayos en el primer grupo: a) ensayos de tipo "screening", donde se incluyen los ensayos diseñados para obtener una medida inicial de la toxicidad; b) ensayos desarrollados sobre la fase líquida de los sedimentos, tanto agua intersticial como lixiviados, diseñados para simular los efectos en la columna de agua por la resuspensión y sedimentación de los sedimentos durante las actividades de dragado y c) ensayos realizados sobre la fase sólida, diseñados para evaluar los efectos sobre organismos bentónicos que viven directamente en contacto con el sedimento en bruto. Posteriormente fueron clasificados según su estado de desarrollo y según la posibilidad de ser utilizados en España para la caracterización de materiales de dragado y se identificaron las especies más adecuadas para ser utilizadas en cada ensayo (DelValls et al., 2001).

3.1. Fases de desarrollo a seguir para el desarrollo de nuevos bioensayos de toxicidad para ser usados en un contexto regulador (según Dillon, 1994).

Fase I: Desarrollo inicial	**Fase II. Evaluación por múltiples laboratorios**	**Fase III. Desarrollo de un método estándar**	**Fase IV. Evaluación por grupos de usuarios**
i. Ámbito/competencia	*Continuo desarrollo e investigación*	*Comparaciones intertest*	Evaluación conjunta de las agencias
Fundamento	Estudios interlaboratorio	Verificación/validación	Formación con "receta" institucional
Medidas de evaluación	Guía de interpretación	Publicaciones peer-reviewed	Recomendaciones de las agencias
Medidas finales	*Prueba con más materiales de dragado*	Protocolo publicado por grupos de estandarización	Revisión periódica
Especies de ensayo	*Sensibilidad de las especies a contaminantes de interés*		
Revisión bibliográfica	*Coste y logística*		
Protocolo alfa	Publicaciones peer-reviewed		
ii. Investigación en el laboratorio	Aceptación por la comunidad científica		
Diseño estadístico	Protocolo gamma		
Diseño experimental y procedimiento			
Calidad/control			
Robustez del ensayo			
Ensayo con materiales de dragado			
Publicaciones peer-reviewed			

En una segunda fase se seleccionaron los ensayos más convenientes para ser sometidos a una fase de prueba en el laboratorio con materiales de dragado. Los ensayos seleccionados para esta fase fueron los siguientes:

1) El ensayo de inhibición de la luminiscencia con una población de bacterias *Vibrio fischeri*. Este ensayo fue seleccionado por ser un ensayo de tipo comercial recomendado por numerosas agencias nacionales e internacionales para la caracterización de materiales de dragado en un contexto regulador. Aunque se han identificado algunos factores de confusión para la interpretación de los resultados parece ser uno de los ensayos más prometedores como herramienta de evaluación inicial, lo que se denomina con el término inglés de "screening".

2) El ensayo sobre la fase sólida con anfípodos. Este ensayo ha sido también recomendado por numerosas agencias internacionales y nacionales y está ampliamente establecido en los laboratorios españoles, que lo han utilizado de forma rutinaria para la evaluación de la calidad de sedimentos costeros. En este caso se han comparado organismos de las especies *Corophium volutator* y organismos de la especie de interés regional *Ampelisca brevicornis*.

3) El ensayo sobre la fase sólida con poliquetos de la especie *Arenicola marina*, recomendado en el Reino Unido para la caracterización de este tipo de muestras.

4) El ensayo sobre la fase sólida con erizos irregulares *Echinocardium cordadum*, estandarizado en Holanda para dragados portuarios.

5) El ensayo sobre la fase sólida con juveniles de almeja de la especie *Ruditapes philippinarum*, especie de interés comercial en España y que ha sido ampliamente usado para la evaluación de la calidad ambiental de sedimentos costeros.

6) El ensayo sobre la fase líquida con una población de rotíferos de la especie *Brachionus plicatilis*. Este ensayo se desarrolla con una población completa de individuos expuestos durante 7 días con lo que se evalúa una medida de tipo crónico.

7) El ensayo sobre la fase líquida de desarrollo larvario con embriones del erizo de mar *Paracentrotus lividus*. Este ensayo está ampliamente establecido en España y ha sido usado rutinariamente para caracterizar sedimentos costeros. Además está recomendado por las agencias Canadiense y Americana para la evaluación de la toxicidad de los lixiviados de materiales de dragado.

Tabla 3.2. Inventario de bioensayos usados para la evaluación de la calidad de sedimentos y/o dragados portuarios.

Bioensayo	Especie	Medida final	Ruta de exposición (tipo)	Tiempo de exposición	Guías	Referencias
Microtox® (bacteria)	*Vibrio fisheri*	Inhibición de la bioluminescencia (IC_{50})	Screening (lixiviado, agua intersticial, fase sólida)	5-30m	ASTM, EPS, RIKZ, NOAA, APHA, ISO, DIN	RIKZ, 1999; EC, 2002; ISO, 1997; APHA, 1995; ASTM, 1995.
Rotoxkit® (rotíferos-neonatos)	*Brachionus plicatilis*	Supervivencia (LC_{50})	Screening	24 h	RIKZ	RIKZ, 1999a.
Pseudomonas sp.	*Pseudomonas fluorescense.*	Actividad enzimatica	"	48 h		
Copépodos	*Oncaea sp., Euterpina sp., Acartia clausii* (muestras estuáricas)	Supervivencia (LC50)	Fase líquida	48h	RIKZ, CEFAS, ISO	ISO, 1999; CEFAS, 1997; RIKZ, 1994a 1994b;
Fitoplancton	*Tetraselmis sp, Nannochloropsis gaditana, Chaetoceros sp., Chlorella sp.*	Crecimiento	"	2-5d	OECD, ISO, APHA-AWWA-WPCF	OECD, 1998a; ISO, 1995; APHA-AWWA-WPCF, 1992.
Fertilización y desarrollo larvario	*Paracentrotus lividus*	Fertilización (%), desarrollo.	"	48h/ 96h	EPA, CETESB, OECD, RIKZ	RIKZ, 1999b; CETESB, 1999; US EPA, 1996, 1995; ASTM, 1986.
Población de rotíferos	*Brachionus plicatilis*	Decrecimiento poblacional (LT50)	"	7d		

Tabla 3.2. Inventario de bioensayos usados para la evaluación de la calidad de sedimentos y/o dragados portuarios (Cont.).

Bioensayo	Especie	Medida final	Ruta de exposición (tipo)	Tiempo de exposición	Guías	Referencias
Anfípodos	*Ampelisca sp., Corophium sp., Microdeutopus gryllotalpa*	Supervivencia	Fase sólida	10d	EPA, ASTM, CEFAS, RIKZ, ICES, EPS, PARCOM	EC, 2000; RIZK, 1999d; PARCOM, 1995; US EPA, 1994; ASTM, 1991.
Equinodermos	*Echinocardium cordatum*	Supervivencia, enterramiento	"	14d	RIKZ	RIKZ, 1999c.
Poliquetos	*Arenicola marina, Nereis sp., Neanthes sp.*	Supervivencia, crecimiento	"	10d/ 14d	PARCOM, ASTM, CEFAS	PARCOM, 1995; ASTM, 1994; Thain & Bifield, 1993.
Misidaceos	*Neomysis integer, Siriella armata*	Survival	"	10d	EPA, ASTM	US EPA, 1993; 1987; ASTM, 1990.
Microalgas bentónicas	*Cylindroteca closterium*	Crecimiento	"	72 h		
Bivalvos	*Ruditapes philippinarum, Scrobicularia plana, Venerupis pullastra, V. rhomboideus, Tapes decussates.*	Supervivencia, enterramiento, biomarcadores, histología	"	7d	EPA	USEPA, 1995.
Peces pelágicos y bentónicos	*Solea senegalensis, Scophtalmus maximus, Sparus aurata, Fundulus heteroclitus*	Supervivencia, enterramiento, biomarcadores, histología	"	96h-60d	PARCOM, OECD, EPA, ICES	ICES, 2000; PARCOM, 1995; US EPA, 1995; OECD, 1992.

Tabla 3.2. Inventario de bioensayos usados para la evaluación de la calidad de sedimentos y/o dragados portuarios (Cont.).

Bioensayo	Especie	Medida final	Ruta de exposición (tipo)	Tiempo de exposición	Guías	Referencias
Bivalvos	*Ruditapes philippinarum, Scrobicularia plana*	Bioacumulación (BCF)	Todas	28d	EPA, OECD	OECD, 1998b, ASTM, 1997.
Poliquetos	*Arenicola marina, Neanthes sp., Nereis sp.*	Bioacumulación (BCF)	Todas	28d	EPA, ASTM	US EPA, 1993; ASTM, 1997.

En este capítulo se presentan los resultados de la aplicación de estos ensayos sobre las muestras de los materiales de dragados caracterizados en el capítulo anterior. Se presentan los resultados de cada uno de los bioensayos de forma conjunta con los resultados de la caracterización físico-química. El objetivo es establecer posibles relaciones entre contaminación y toxicidad, estudiar posibles factores de confusión identificados mediante el tamaño de grano y contenido en materia orgánica del sedimento y, considerando los NNAA en uso, estudiar el uso de cada uno de los ensayos utilizados en el marco de gestión para dragados portuarios.

Así, en el trabajo III se muestra que el ensayo con anfípodos de la especie *Corophium volutator* es mucho más sensible que el ensayo con poliquetos de la especie *Arenicola marina*, con una respuesta positiva de toxicidad del 80 y 25% respectivamente para un total de 16 muestras consideradas. La incidencia de toxicidad de materiales de Categoría I es del 0% para ambos ensayos, mientras que la incidencia de toxicidad para materiales de Categoría III es del 91 y 36% respectivamente, dependiendo del bioensayo empleado. En cuanto a los materiales de Categoría II, para los que serían de aplicación los ensayos biológicos para la toma de decisiones, la incidencia de toxicidad está entre el 75 y 0% respectivamente.

Existe una mayor correlación entre la contaminación medida en los sedimentos y los resultados del ensayo de toxicidad con anfípodos, aunque también es mayor la correlación entre la mortalidad del anfípodo *C.volutator* y la proporción de finos y el contenido en materia orgánica del sedimento (significativa a $p<0.05$ y $p<0.01$, respectivamente). Esto se explica por las distintas preferencias de alimentación de uno y otro organismo, ya que el anfípodo *C.volutator* se alimenta sobre la fracción <63 μm, que es la fracción sobre la que se realizan los análisis químicos, mientras que el poliqueto *Arenicola marina* se alimenta de la fracción <500 μm aunque evita las partículas más finas, con mayor capacidad de incorporación de contaminantes y de materia orgánica. Esto explicaría también la distinta sensibilidad de estos dos bioensayos.

La comparación entre las especies de anfípodos *C.volutator* y la especie de importancia regional *Ampelisca brevicornis* (trabajo IV) mostró que ambas especies respondieron con sensibilidad similar a materiales de dragado de puertos españoles con una incidencia de toxicidad del 72 y 68% respectivamente para un total de 22 muestras consideradas, aunque se encontró un 10% de muestras para las que el resultado de toxicidad no coincidían con la categoría establecida mediante los análisis de contaminación. Los resultados de este estudio mostraron un valor añadido en el uso de especies de anfípodos autóctonas de la zona a

dragar, ya que la sensibilidad puede ser diferente en el caso de sedimentos de contaminación intermedia. Estos resultados podrían estar causados por una mayor sensibilidad debida a la manipulación de los individuos sometidos al envío desde otro lugar cuando no están disponibles en el laboratorio de análisis.

El ensayo comercial con bacterias luminescentes Microtox® SP apareció como el más sensible de todos los ensayos realizados sobre la fase sólida (trabajo V), con un 80% de toxicidad sobre un total de 19 muestras analizadas si se considera el criterio de calidad establecido por la agencia canadiense de 1000 mg/L expresado en peso seco. De acuerdo con estudios previos se han identificado importantes factores de confusión a la hora de interpretar los resultados relacionados con la granulometría del sedimento. En este sentido, los valores de toxicidad pueden incrementarse decisivamente en caso de altas proporciones de finos en el sedimento dando lugar a los denominados falsos positivos. En cualquier caso, en un contexto regulador, parece preferible un número razonable de falsos positivos que puedan ser identificados como tal en posteriores investigaciones que un alto número de falsos negativos de toxicidad utilizados para permitir el vertido al mar de materiales de dragado potencialmente nocivos. En este caso, se han identificado falsos negativos de toxicidad aunque este tipo de errores no son de importancia al ser correctamente identificados mediante el análisis químico de los sedimentos.

En cuanto al posible establecimiento de guías ecotoxicológicas para la interpretación de este bioensayo con el fin de evaluar la calidad de sedimento, existe una gran variabilidad en los criterios establecidos por las distintas agencias que incluyen este bioensayo como herramienta de gestión. El criterio canadiense es el que ofrece los resultados más satisfactorios. No se ha considerado el desarrollo de ningún criterio para la corrección de los datos según la proporción de finos en las muestras ya que las modificaciones estudiadas resultaban difíciles de interpretar. Parece recomendable la comparación de los resultados con un sedimento de referencia para las muestras con una proporción elevada de arenas; para las muestras con una gran turbidez y color se está valorando la corrección desarrollada por Campisi et al. (2005).

Como se muestra en este mismo trabajo, el ensayo de toxicidad sobre la fase sólida del sedimento con el erizo irregular *Echinocardium cordatum* parece ser menos recomendable para su aplicación en un contexto regulador, aunque los resultados son satisfactorios. Su sensibilidad a los materiales de dragado es superior a la de otros organismos bentónicos utilizados, como por ejemplo el poliqueto *Arenicola marina* (un 50% de las muestras fueron consideradas tóxicas según el erizo frente al 25% resultante del ensayo con poliquetos). Se

encontraron dificultades para recolectar el número de organismos necesarios mediante muestreos de campo, lo que supone una potencia estadística menor así como un encarecimiento de los costes.

De forma similar, el ensayo con juveniles de almeja resultó el menos sensible de todos los ensayos realizados para evaluar la toxicidad de la fase sólida del sedimento. Este organismo, que está en contacto directo con el sedimento ya que se encuentra enterrado en él, está expuesto a la contaminación principalmente a través del agua superficial filtrada por sus sifones, por lo que la ruta de exposición es distinta a la de los otros ensayos utilizados. Aunque este ensayo parece adecuado para su introducción en un contexto regulador, ya que cumple con las características imprescindibles, se recomienda su uso sólo en los casos en que este tipo de organismos bentónicos filtradores sean específicamente organismos en riesgo y siempre teniendo en cuenta su menor sensibilidad respecto a otro tipo de organismos bentónicos como pueden ser los anfípodos, poliquetos o erizos. En cuanto a las dos medidas subletales consideradas, el porcentaje de enterramiento de erizos tras 24 horas de exposición ofreció resultados más satisfactorios que el ensayo con almejas, ya que este último sólo fue sensible a los materiales de dragado de un puerto en concreto, afectado por contaminación por metales, pero no la más elevada. Esta medida parece más afectada por la contaminación de las fases de exposición líquidas.

Como se muestra en el último trabajo de este capítulo, de entre los dos ensayos diseñados para evaluar la toxicidad sobre organismos pelágicos producida por los lixiviados de los materiales de dragado, el ensayo con larvas del erizo es más sensible que el ensayo con rotíferos. Este último ensayo se ve afectado por procesos de estimulación debido a las altas concentraciones de nutrientes en los lixiviados de materiales de dragado, lo que provoca un elevado porcentaje de muestras que ofrecen resultados positivos. Aunque los resultados del ensayo con larvas de erizo pueden verse afectados críticamente por la presencia de compuestos naturales del sedimento, como pueden ser el sulfuro o el amonio, la presencia de estos factores de confusión puede ser fácilmente detectados, y por el contrario, parece ser un buen indicador de la toxicidad por exposición a las fases líquidas del material de dragado.

Por último, la comparación de los resultados obtenidos para los ensayos en fase líquida y los ensayos en fase sólida evidenció la ausencia de correlación entre la contaminación en los sedimentos y la toxicidad por exposición a los lixiviados. Esto confirma que los análisis químicos del sedimento no pueden ser utilizados para identificar la toxicidad producida por la resuspensión de este tipo de materiales durante su dragado y

posterior vertido, especialmente para los contaminantes de tipo orgánico. De acuerdo con estos resultados es recomendable el uso de una batería que incluya ambos tipos de ensayos para una completa caracterización de los materiales de dragado, ya que no siguen una tendencia común sino más bien ofrecen resultados complementarios.

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Comparative toxicity assessment using the amphipod *Corophium volutator* and the polychaete *Arenicola marina* for dredged material management[1]

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ABSTRACT: The polychaete *Arenicola marina* and the amphipod *Corophium volutator* are reference methods for sediment and dredged spoil toxicity assessment. The present study was conducted to evaluate these sediment toxicity tests as potential tools to characterize dredged material in Spain according to the current system of authorisations for sea disposal. In this context dredged sediments from different Spanish ports were characterised according to Spanish recommendations for dredged material management and toxicity was assessed following standard operational procedures (SOPs) for these two assays. Both tests were feasible and results were related to sediment chemical concentrations, specially amphipod mortality that reported significant correlations with the metallic compounds As, Cd, Cu, Hg, Pb and Zn. According to these results the selection of one or the other species for dredged material toxicity assessment foresees different dredged material classification and different management options. Possible consequences of such results in dredged material assessment framework are further discussed.

Keywords: ecotoxicology, whole-sediment toxicity, test battery, lugworm

New approaches to effectively characterize dredged materials before the best management option is selected are focused on the use of toxicity bioassays due to the complexity of environmental matrix. Sediment bioassays seem the most suitable measure of the actual bioavailability of contaminants and take into account sediment characteristics and all possible interactions between the present chemical species. Moreover the use of the traditional chemical characterization and the sediment quality guidelines (SQGs) used to classify materials should only be used as initial screening tools in early stages of sediment characterisation (GIPME, 2000). Although sediment quality assessment have been further

[1] Environmental Toxicology (Aceptado)

used in Spain (i.e. Mariño-Balsa et al., 2003; Riba et al., 2004) the use of bioassays for dredged material management is still on an early stage. The objective of this study was to compare two standardised solid phase bioassays for dredged material toxicity characterisation in Spain: the one using the amphipod *Corophium volutator*, an active suspension and surface deposit feeder that occupies semi-permanent U-shaped burrows in the fine sediments of mud flats, and the bioassay using the polychaete *Arenicola marina*, also a surface deposit feeder feeding on the organic fraction of the just-sedimented detritus of the ingested sediment.

The sediment samples were collected at 4 commercial ports along the Spanish coast (Cartagena, Barcelona, Huelva and Cádiz named C#, B#, H# and CA# respectively) to a total of 16 stations and were characterized for the physico-chemical properties according to Spanish recommendations for dredged materials (CEDEX, 1994). A more complete study on these sediments have been previously reported (Casado-Martínez et al., 2006). Generally the samples consisted of muddy sediments (percentage silt and clay ranging from 31% to 99.59%) with high organic matter content. The chemical data showed that most of the samples contained mixtures of contaminants, including trace metals, PAHs, and chlorinated hydrocarbons. The concentration of most trace metals ranged from two to three orders of magnitude and even four for Hg. The highest concentrations for most of the metallic compounds were found in Cartagena and Huelva. PCBs were not detected for most of the samples at the ports of Cádiz and Huelva and were higher in Barcelona and Cartagena. According to the recommendations for dredged material management (CEDEX, 1994) and the SQGs used (Action Levels), samples CA1 and H4 could be freely disposed since no potential effects are expected. Samples CA3, CA4 and B1 and B3 would need of further assessments due to its intermediate grade of contamination while the rest of samples (more than 60 %) could not be suitable for aquatic disposal since Action Level 2 is exceeded for some compounds.

For the bioassay using amphipods individuals of *Corophium volutator* were collected in a clean intertidal area (DelValls et al., 2003) and were shipped to the laboratory (shipping time was about 24 hours). Once in the laboratory the organisms were maintained for acclimatization to test conditions (temperature 15ºC and salinity 35) for 7 days before being used in experiments. The bioassay followed standard operational procedures (ASTM, 1990; U.S.EPA, 1994; RIKZ, 1999). A negative toxicity control, consisting on sediment from the area where animals were collected, and a positive toxicity control consisting of a solid

dilution of toxic mud from a mining spill (Riba et al., 2003) were carried out with the tested sediments. Each treatment comprised three replicates.

Lugworms were collected in the Cantabric coast (north of Spain) by hand digging and were shipped refrigerated to the University of Cádiz in clean seawater. The time between the collection and the arrival was about 24 hours and the shipping time was no more than 12 hours. Once in the laboratory the organisms were maintained for acclimatization to test conditions about one week before the tests (temperature 15ºC and salinity 35). The bioassay followed protocol by Thain and Bifield (2001). Briefly, five organisms were used per replicate and three replicates were used per treatment. On the first 24 hours of the experiment test chambers were carefully observed for burrowing activity even though it was difficult due to the fine texture of the sediments and resuspension. A negative toxicity control consisting on sediment from the area where animals were collected, and a positive toxicity control (same than for amphipods) were carried out with the tested sediments. Since parametric statistical analyses could not be used the Fisher test was used to determine statistically significant toxicity for both amphipod and polychaete tests ($p=0.05$). Analyses were developed using the 'Simple Interactive Statistical Analysis' (SISA), available on http://home.clara.net/sisa/. The relative incidence of toxicity was compared by calculating the percentage of samples that were found to be significantly toxic for each test and differences in sensitivity between amphipod and lugworm survival were also compared by normalizing to control values, then comparing these values for each sample tested (Anderson et al., 1998). Spearman rank correlations were used to determine associations between toxicity tests results, contaminant concentrations and other sediment parameters. Correlation coefficients were developed using the statistical program STATISTICA® 5.1.

All the tests met quality assurance/quality control (QA/QC) requirements. Test parameters were among the range to ensure good water quality and survival for the control sediments were above the critical value for test acceptability (90%) for both bioassays. Relative incidence of toxicity was much higher for the bioassay using *C.volutator* than for *A.marina*, with 80 and 25% of samples considered toxic respectively (Fig. 1). These results are in accordance with previous results reported by Anderson and colleagues (1998) for the amphipod *Rhepoxynius abronius* and the growth inhibition test using the polychaete worm *Nereis arenaceodentata* of 71% and 20% respectively using a database of ca. 300 harbour sediments from the California state (CA, USA), and with those reported on sediments from Pudget Sound (WA, USA) using either *R.abronius* and *Eohaustorius estuarius* and

N.arenaceodentata (Pastorak and Becker, 1990). No information was available from direct comparison studies for the 10d bioassay using *C.volutator* and *A.marina* even though relative sensitivities to specific metallic contaminants have been reported previously (Bat and Raffaelli, 1998). LC_{50} for *Corophium volutator* was 14 μg g^{-1} for Cd, 37 μg g^{-1} for Cu and 32 μg g^{-1} for Zn while *Arenicola marina* reported 35 μg g^{-1} Cd, 20 μg g^{-1} Cu and 50 μg g^{-1} Zn in spiked sediments thus being the amphipod much more sensitive to Cd and Zn and contrary less sensitive to Cu than the polychaete. Bioavailability of spiked metals depends on a wide number of sediment physico-chemical properties that will ultimate determine the actual exposure concentrations since they determine the quantity and the form in which contaminants bound to sediment particles.

Mortality of *Corophium volutator* showed statistical significant correlations with Cu, Zn, Cd, Hg and As ($p<0.01$) and to a lesser extend with Pb ($p<0.05$) (Table I) while polychaete mortality was correlated with Cu, Ni and Zn (at $p<0.05$). Similarly lethal effects in amphipods were more correlated with the proportion of fines and the organic content. This correlation could be related to the different feeding strategies of these two organisms since *Corophium volutator* feeds on the fraction between 4 and 63 μm and *A.marina* prefers the fraction lower than 500 μm, which could explain the higher correlation between *C.volutator* mortality and the sediment chemical concentrations that were actually measured in the fraction <63 μm. Nonetheless the higher contamination of sediment particles <63 μm, feeding preferences and sediment selection could also be determinant in the lower sensitivity of *A.marina* and in the misfit between toxic effects and sediment chemical concentrations. Environmental samples are characterized by its complex nature, with an unknown number of contaminants and unknown synergistic and antagonistic effects, thus sediment concentrations would not be indicative of potential toxicity in laboratory bioassays.

For a better evaluation of dredged materials laboratory bioassays are usually integrated in a more complete characterization process that usually follows a tiered approach, including the physico-chemical characterization in the first tiers followed by further toxicity assessments if necessary. Our results reported no toxicity neither using *C.volutator* nor *A.marina* for low priority sediments (CA1 and H4) although these sediments would be excluded of further assessments according to their coarser nature (CEDEX, 1994). For samples classified in category II and actually the only sediments that would need of toxicity testing to decide whether they are suitable for open water disposal or not under Spanish recommendations (samples CA3, CA4, B1 and B3), the results differ depending on

the selected test species. Overall toxic responses followed similar trends but the use of the amphipod bioassay would consider category III all sample stations except B1, which reported intermediate Hg and PCBs concentrations and the lower contamination of all sediments from Barcelona. Contrary the polychaete would classify all category II materials as not toxic thus in category I.

TABLE I. Spearman rank correlation coefficients for the contaminants of concern and amphipod and lugworm mortality.

	Spearman rho	
Toxicant	***Corophium volutator***	***Arenicola marina***
% fines	0.567*	0.085
% TOC	0.635**	0.006
Metallic compounds		
As	0.647**	0.484
Cd	0.673**	0.344
Cr	0.224	0.296
Cu	0.741**	0.507*
Hg	0.668**	0.281
Ni	0.486	0.561*
Pb	0.612*	0.352
Zn	0.718**	0.535*
Organic compounds		
PCBs	0.328	-0.106
PAHs	0.218	-0.003

* = significant at 0.05; ** = significant at 0.01.

The polychaete reported toxicity at stations H1, H3, C1 and C3 (Fig. 1) it is 25% of the total number of samples. H1 reported the highest concentrations of all chemicals in sediments from Huelva except PCBs and Ni, which found the highest value at H3 and could be responsible for the higher toxicity for this sample. Similarly samples C2 and C4 were not considered toxic by the polychaete bioassay despite its classification in category III. Both samples reported high concentrations of some metals (especially Cd and Hg) and reported the highest concentrations of organic compounds (PCBs failed the upper limit value). Nevertheess higher toxicities were related to higher sediment contamination thus it seems probable that bioavailability of metals is lower in this area compared to other areas under study.

From test results it could be inferred that 60% of the samples reported control-normalized amphipod mortality higher than control-normalized lugworm mortality. Contrary 30% of sediments had control-normalized lugworm mortality higher than amphipod control-normalized mortality. Thus in addition to the lower sensitivity of

A.marina, the use of a lower number of organisms per treatment can determine a higher minimum difference to consider a sample as toxic although it could be easily solved by increasing the statistical power by increasing the number of organisms per treatment for future tests. These organisms usually dominate benthic habitats, are key ecological constituents of benthic assemblages and are burrowers and deposit-feeders therefore contaminant exposure is likely (Thain and Bifield, 2001). Amphipods have some technical advantages since they are found in very high densities and little effort is needed to collect them, are small and need of less space in the laboratory and less volume of sediment thus a higher number of samples can be tested at the same time. Despite this is a first approach to dredged material toxicity assessment the results showed important differences in the relative sensitivity of these two species to environmental sediment samples and major differences in the classification of dredged materials depending on the test organism. The amphipod was by far more sensitive than the polychaete but there is a higher uncertainty due to potential confounding factors such as the proportion of fines and the organic content, which could in turn determine the higher exposure to contaminants due to feeding preferences and particle selection. Limit values for some chemicals have been already established but the applicability of such values in sediment toxicity assessment seems limited given the higher proportion of sediments failing these toxicity criteria and the different bioavailability depending on the sediment properties. In this sense the use of reference sediments and control sediments with each treatment can reduce the probability of false positives and improve significantly the performance of this test in decision-making.

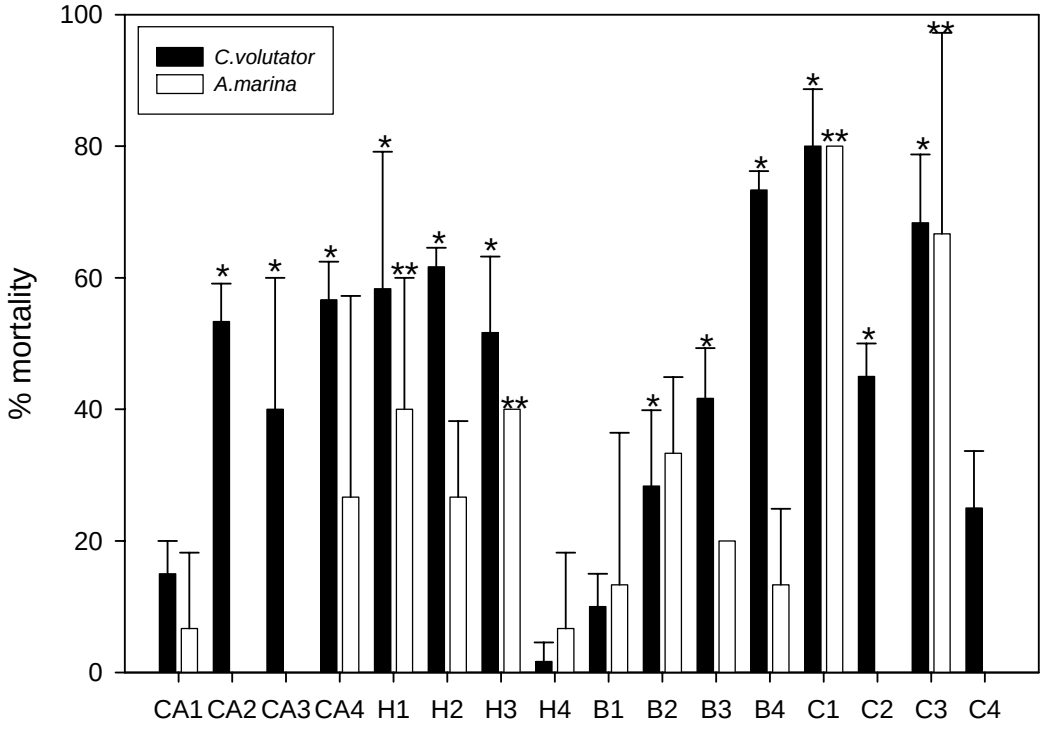

Fig. 1. Mean mortality of *Corophium volutator* and *Arenicola marina*. Each value is the mean of the three replicates (* and ** means significantly different from control sediment at p=0.05).

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Direct comparison of amphipod sensitivities to dredged sediments from Spanish ports.[1]

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Abstract

The sensitivity of the benthic amphipod species *Ampelisca brevicornis* and *Corophium volutator* to dredged sediments were compared through simultaneous testing on the standard 10-d sediment toxicity test. Both species reported similar incidence of toxicity for medium-high and highly contaminated sediments but it seems that *C.volutator* may be more sensitive to low and medium-low contaminated sediments. Both species reported higher sensitivities than other amphipod species used for whole-sediment toxicity testing but the results were still in the range of previous studies. *A.brevicornis* mortality presented the highest correlation with the metallic contaminants while *C.volutator* was more correlated with the organic micro-pollutants and the sediment properties total organic matter content and proportion of fine sediments. In general, amphipod mortality was better predicted through the use of mean quotients than just evaluating the contamination by comparison with the single sediment quality guidelines. Nonetheless, the results indicate that both species are suitable for dredged material characterisation.

Keywords: dredged material characterisation, test battery, bioassay.

1. Introduction

In the late centuries, the multiple anthropogenic activities that take place in ports, harbours and other navigational channels have introduced a wide number of contaminants that ultimate accumulate in the sediments. The large volume of sediments remobilized during dredging activities may represent an important source of pollution to the environment if high concentrations of contaminants are present, which have provoked its

[1] Chemosphere (Enviado)

regulation by a number of global and regional conventions for the prevention of marine pollution.

Decision-making in dredged material management has been traditionally based on the results of the chemical analyses and the comparison with the content in unpolluted sediments (background concentrations) or with a set of limit concentrations established for different purposes (GIPME, 2000). There are evidences of the unsuitability of this chemical approach for predicting the toxicity of chemically and physically complex matrix such as dredged sediments (Crane, 2003; McCauley et al., 2000; O'Connor et al., 1998), and at present bioassays are also recommended for the characterization of the toxic potential of dredged material and for the environmental risk assessment of disposal activities (LC, 1996; GIPME, 2000; PIANC, 2006). Spain does not have a nation-wide regulation for the management of dredged material. The "Recommendations for Dredged Material Characterization" were set up in 1994 and have been widely applied (den Besten et al., 2003). Bioassays are recommended for medium contaminated sediments but they have been hardly applied due to the absence of routine methodologies (CEDEX, 1994). Several marine bioassays are already established in Spain and are standardized to different degrees (DelValls et al., 2003) but judging the suitability of a particular test for an application should be based on the quality and quantity of scientific information available regarding the test itself and its application with field-collected sediments (PIANC, 2006).

The bioassay using crustacean amphipods is widely used for whole-sediment toxicity assessment. Amphipods are among the first species to disappear from benthic marine communities in contaminated areas and are sensitive indicators for sediment pollution with ecosystem relevance. They are abundant and ecologically important components of soft-bottom estuarine and marine benthic communities; they are widely distributed, live in direct contact with the sediment, are tolerant to a wide range of different environmental variables and seem to be suitable for handle and culture. Standard protocols already exist for some species including test conditions, quality assurance/quality control guidance and reference standards to ensure that the organisms being used in the test are of sound health (ASTM, 1991; EC, 1992; RIKZ, 1999; US EPA, 1994). The U.S EPA recommends the species *Rhepoxynius abronius* and *Ampelisca abdita* while *R.abronius, Eohaustorius washingtonianus*, *E. estuarius* and *Amphiporeia virginiana* are preferred in Canada (EC, 1992). In Europe *Corophium volutator* is chosen by the OSPAR as standard sediment reworker test for testing off-shore chemical/products (OSPARCOM, 1995) and is also recommended in the Netherlands, United Kingdom and Belgium for contaminated sediments and dredged material

characterisation (den Besten et al., 2003). Nonetheless this species is not always available thus, despite *Corophium volutator* is preferred, other species available locally have been used in the same context and may be suitable where "routine, standard test methodologies already exist" (SETAC, 1993 cited in Costa et al., 1998).

In Spain the species *Corophium volutator* and *C.multissetosum* have been proposed as test organisms in the Northern coasts (Belzunce et al., 2004) and *Gammarus aequicauda* and *Microdeutopus gryllotalpa* has been used in the Mediterranean (Cesar et al., 2002; DelValls et al., 1998). Similarly, *Ampelisca brevicornis* has been widely applied in the Atlantic coast for contaminated sediment assessment (Riba et al., 2003), but there is still few information regarding the application of these new species with dredged sediments. In this study we conducted the 10 day bioassay using crustacean amphipods of the species *Ampelisca brevicornis* and *Corophium volutator* on dredged sediments from Spanish ports. The main objectives were to study the relative sensitivity of the two amphipod species for dredged material toxicity assessment and to evaluate if this test provide with meaningful and reliable information in addition to the traditional chemical guidelines.

2. Material and methods

2.1. Sediment sampling and characterization

22 sediment samples were tested, all collected at different ports around the Spanish coast. The sediment characterization followed the Spanish recommendations for dredged materials (CEDEX, 1994). Grain size distribution followed UNE 103 101 and total organic carbon (TOC) content was measured by loss of ignition (LOI) at 550ºC as recommended for small dredged volumes. Metals were determined in microwave acid-digested samples. For Hg the cold vapour technique was used and for As hydride generation, and both quantified using atomic absorption spectrometry. Cd, Pb, Cu, Zn and Cr concentrations were determined using flame or furnace atomic absorption spectrometry, depending on the metal content. PCB congeners #28, 52, 101, 118, 138, 153 and 180 and polycyclic aromatic hydrocarbons (PAHs) were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment and concentration and clean-up with column chromatography. Determination of PCBs was made with gas chromatography with electron capture detection (GC-ECD) (EPA 8080) and PAHs, not included in the recommendations, were determined with HPLC with fluorescence detection (EPA 8310). All the analytical procedures were checked with reference material (Conceil National de

Reserches Canada, 277 BCR, for heavy metals; and NRC-CNRC HS –1 for organic compounds) and allow agreement with certified values higher than 90%.

The results of the chemical characterization were compared with the national limit values used for dredged material management (CEDEX, 1994; Casado-Martínez et al., 2005). For comparison purposes the results of the single chemicals were used to develop mean quotients for the whole set of chemicals following the approach developed by Fairey et al. (2003) and Long and MacDonald (1998).

2.2. Toxicity tests

On arrival to the laboratory sediments were sieved through a 0.5 mm mesh to avoid any interference during test development and stored at 4ºC and darkness prior to analysis. Bioassays started within two weeks after sediment sampling. Individuals of the species *Ampelisca brevicornis* were collected from a pristine area located in the Bay of Cádiz (DelValls et al., 1997) by sieving through a 0.5 mm mesh. They were brought to the laboratory in aerated clean sea water and were acclimated for less than one week to laboratory conditions. The individuals of *Corophium volutator* were collected by sieving through a 1 mm mesh at Oesterput in the Ooesterschelde estuary located in the Netherlands and then shipped and received in the laboratory in Spain in less than 48 hours. Organisms were acclimated to laboratory conditions for two weeks after arrival to our laboratory and prior to toxicity testing.

Toxicity tests were carried out following standard procedures (e.g. ASTM, 1991; US EPA, 1994). Briefly, 2 litres glass beakers were filled with a 3 cm layer of sediment (approximately 250 mL) and clean sea water (1000 mL) in a proportion 1:4 v/v. Test chambers were left to settle and then aerated for 12 hours prior to the addition of test organisms. 20 animals were randomly selected and added to each replicate and three replicates were used for each sediment sample. During the exposure period aeration was adjusted to ensure water quality requirements but not to disturb the sediment surface. Water quality parameters were measured before and at the end of the bioassay. Test parameters and conditions are summarised in Table 1.

The Fisher test was used to determine statistical significant differences ($p=0.05$) between the toxicity registered for each sediment sample and the negative toxicity control (here CA1) using the 'Simple Interactive Statistical Analysis' (SISA), available on http://home.clara.net/sisa/.

Table 1

Parameters and conditions followed for the dredged material toxicity tests.

Parameter	**Conditions**
1. Test type	Static. Whole sediment
2. Temperature	18±1ºC
3. Salinity	35
4. Light quality	Wide spectrum fluorescent light
5. Illumination	500-1000 lux
6. Photoperiod	Natural
7. Test chambers	Cylindrical, 1 gallon
8. Volume of sediment	200-300 mL
9. Volume of overlying water	800-1200 mL
10. Water renewal	No
11. Size of organisms	*Ampelisca* 3-5 mm *Corophium* higher than 5 mm
12. Organisms per test chamber	20
13. Number of replicates	3
14. Feeding	No
15. Aeration	12 hours before addition of organisms and during the exposure to ensure equal or higher than 90%
16. Overlying water	Clean sea water
17. Water quality	Temperature, pH, ammonia, salinity and dissolved oxygen at the beginning and at the end of exposure.
17. Test duration	10 days
18. Endpoints	Survival
19. Test acceptability	Minimum survival of 90% in the control sediment

3. Results

3.1. Traditional sediment characterization

Multiple contaminants exceeded national numerical sediment quality guidelines (Table 2). Three samples were classified in Category I according to their low chemical contamination: a sandy sediment (CA1), a coarse sediment (H4) and a fine sediment (BI3). Only four samples were classified in category II with intermediate level of contamination: two samples only exceeded the lower limit value for Cd (CA3 and CA4) nd two samples due to the Hg, Cu and PCBs concentrations (B1 and B3). Sixteen samples failed at least one of the higher limit values for open water disposal authorization: 12 samples failed AL2 for PCBs, AL2 for As and Cu was failed for 6 samples, and Hg, Cd, Pb and Zn for 5, 4, 3, and 2 samples, respectively. Concentrations of PAHs were less than the analytical detection limits in most samples and the highest concentrations were registered for samples BI1 and BI2.

3.2. Toxicity tests

Mean survival in the negative controls was 1.67% for *A.brevicornis* and 15% for *C.volutator*. Replicate precision using *Ampelisca brevicornis* was higher than using *Corophium volutator,* with a total of 3 and 8 samples respectively with relative standard deviation higher than 25%. The criteria to consider a sample as toxic or not toxic according to the obtained results was the statistical difference compared with the negative control (at p=0.05). The number of samples classified as toxic in amphipod survival tests was 15 for *A.brevicornis* and 16 for *C.volutator*, that represent an incidence of toxicity of 68% and 72%. Even if this percentage is similar to that obtained by the traditional chemical classification (68%) a misfit between chemical contamination and toxicity of around 10% was reported when using both amphipod species.

Following a similar approach to that described by Long and MacDonald (1998) and McCready et al. (2005) to test the sensitivity of different amphipod species, sediments were classified as sites of low, medium-low, medium-high and high priority when no AL1 was exceeded and with none, 1 to 3, or more than 3 AL2 were exceeded (Table 3). In the present study 33, 0, 63 and 100% of samples were toxic in *A.brevicornis* survival test when no AL1 exceeded, no AL2 exceeded, from 1 to 3 AL2 exceeded, and more than 3 AL2 exceeded, respectively. The values for *C.volutator* were 0, 75, 59 and 100% (Table 3). The results obtained for medium-high and high priority sites were similar for both amphipod species, with a similar value of 60% of toxic samples when at least 3 AL2 were exceeded (n=12). *Ampelisca brevicornis* reported a false positive according to the chemical results (one sample with all concentrations lower than the corresponding AL1 but considered toxic) while medium low priority sites caused higher mortality to *Corophium volutator*. When the mean quotients are use to classify the sediments the results of toxicity seem to be better predicted (Table 3). *A.brevicornis* mortality increases linearly with increasing priority category, ranging between 16.7 and 78.9% from low to high priority categories, respectively while *C.volutator* mean mortalities ranged from 25 to 64.4%. Similarly the incidence of toxicity increased when increasing the priority category. The use of the mean quotients evidenced the higher sensitivity of *Corophium volutator* to low and medium-low priority sediments, with a difference of 20% registered between the two amphipod species for the low priority sediments while a more similar 50-60% of the medium-low priority sediments were considered toxic for *A.brevicornis* and *C.volutator* respectively.

Table 2

Results of the conventional parameters, chemical characterisation and percentage of mean mortality registered for each of the test species. All concentrations are expressed as mg kg-1 on dry weight basis except PCBs expressed as μg kg-1. Action Levels used for dredged material management options are included for each of the compounds. N.d. means not detected.

	% coarse	**% sand**	**% fines**	**% TOC**	**As**	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**	**PCBs[a]**	**PAHs[b]**	***A.brevicornis***	***C.volutator***
CA1	0.19	99.77	0.04	1.07	3.42	0.92	0.10	6.98	0.05	0.06	2.28	21.27	n.d.	n.d.	1.67	15.00
CA2	0.05	40.42	59.53	13.75	30.77	1.32	14.94	202.80	1.98	20.14	86.90	378.25	144.90	n.d.	48.33	53.33
CA3	0.30	17.80	81.90	20.30	16.61	1.23	8.43	46.76	0.28	16.90	17.61	135.50	n.d.	n.d.	10.00	40.00
CA4	0.03	0.38	99.59	24.33	7.81	1.25	14.22	32.07	0.05	21.25	5.14	65.67	n.d.	n.d.	18.33	56.67
H1	0.07	9.71	90.22	20.27	840.00	4.35	32.89	1938.00	2.38	34.57	383.10	2458.00	2.00	n.d.	51.67	58.33
H2	0.19	9.60	90.21	10.64	531.00	2.50	24.10	1497.00	1.99	7.10	384.70	1857.00	2.29	n.d.	90.00	61.67
H3	0.03	56.02	43.95	6.30	273.00	1.32	8.13	772.00	1.20	129.00	217.60	1176.00	n.d.	n.d.	83.33	51.67
H4	80.34	19.65	0.01	1.00	4.70	n.d.	9.70	1.90	0.04	0.80	5.30	20.90	n.d.	n.d.	11.67	1.67
B1	1.43	64.72	33.86	3.06	17.39	0.93	105.20	74.88	0.94	18.87	86.66	253.80	49.20	0.28	26.67	10.00
B2	5.50	57.92	36.58	4.55	21.19	1.52	103.70	159.70	1.12	29.12	103.50	424.00	138.30	0.37	11.67	28.33
B3	3.89	42.13	53.98	4.81	18.56	0.62	59.53	102.10	1.15	22.24	91.90	219.70	85.30	0.61	23.33	41.67
B4	1.41	39.89	58.70	17.56	28.99	2.88	93.86	601.10	4.12	32.30	455.30	1165.00	272.90	1.80	35.00	73.33
AL1					80	1	200	100	0.6	100	120	500	30	--		
AL2					200	5	1000	400	3	400	600	3000	100	--		

[a] Σ_7-PCBs; [b] Σ_{12}-PAHs.

Table 2

Results of the conventional parameters, chemical characterisation and percentage of mean mortality registered for each of the test species. All concentrations are expressed as mg kg-1 on dry weight basis except PCBs expressed as μg kg-1. Action Levels used for dredged material management options are included for each of the compounds. N.d. means not detected. (Cont.)

	% coarse	**% sand**	**% fines**	**% TOC**	**As**	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**	**PCBs[a]**	**PAHs[b]**	***A.brevicornis***	***C.volutator***
C1	3.95	38.24	57.81	10.54	101.50	98.49	66.64	665.90	136.40	29.04	1397.00	8661.00	123.00	0.91	100.00	80.00
C2	5.22	53.59	41.19	9.12	64.71	17.47	45.61	313.40	32.71	15.33	748.30	1885.00	468.20	1.03	55.00	45.00
C3	0.93	67.20	31.87	7.19	88.00	31.88	57.57	453.30	115.20	19.32	1397.00	3310.00	107.60	0.66	81.67	68.33
C4	0.90	50.01	49.10	9.87	62.55	6.79	29.48	171.10	21.59	19.32	486.70	900.80	118.90	1.24	38.33	25.00
BI1	2.39	20.28	77.33	14.81	67.26	2.00	18.27	102.60	0.74	26.39	147.50	476.10	111.60	66.71	30.00	60.00
BI2	38.12	14.48	47.40	15.07	104.00	2.00	23.11	204.10	1.43	32.00	285.90	777.50	256.20	13.90	81.67	80.00
BI3	0.19	6.22	93.59	16.73	21.71	0.04	3.48	23.03	0.18	15.72	40.70	122.35	22.12	0.63	41.67	11.67
PA1	0.84	28.87	70.29	14.43	39.13	0.68	26.73	158.10	1.07	33.49	293.70	1085.00	610.00	n.d.	65.00	50.00
PA2	3.67	5.08	91.24	18.47	28.86	0.70	23.42	167.10	1.29	28.48	246.00	763.00	740.00	1.06	82.50	91.65
PA3	1.82	38.53	59.65	19.81	23.78	0.04	18.61	162.50	1.36	19.61	154.90	576.00	240.00	0.26	37.70	33.33
AL1					80	1	200	100	0.6	100	120	500	30	--		
AL2					200	5	1000	400	3	400	600	3000	100	--		

[a] Σ_7-PCBs; [b] Σ_{12}-PAHs.

Table 3

Incidence of toxicity of both amphipod species for each priority sediment category and comparison with sediment toxicity data for *Corophium colo* and *R.abronius*.

Contamination category	**Management priority**	**Number of samples in each category**	**% toxicity**				**Mean mortality**	
			A.brevicornis	*C.volutator*	*C.colo*[a]	*R.abronius*[b]	*Ampelisca*	*Corophium*
No AL1 exceeded	Low	3 (13.6)	33.3	0.0	0.0	11.0	18.3	9.5
No AL2 exceeded	Medium-Low	4 (18.2)	0.0	75.0	0.0	15.0	19.6	37.1
1-3 AL2 exceeded	Medium-High	12(54.6)	63.6	59.1	16.0	45.0	54.6	55.6
> 3 AL2	High	3 (13.6)	100	100	28.0	85	78.9	64.5
mAL2q: <0.1	Low	5 (22.7)	20	40	0.0	12	16.7	25.0
mAL2q: 0.11-0.5	Medium-Low	6 (27.3)	50	60	10.0	30	29.6	32.1
mAL2q: 0.51-1.5	Medium-High	8 (36.4)	100	87.5	5.0	46	65.9	61.5
mAL2q: >1.5	High	3 (13.6)	100	100	40	74	78.9	64.4

[a]Data from McCreay et al. (2005)
[b]Data from Long and MacDonald (1998a).

4. DISCUSSION

The major proportion of samples was classified as medium-high and high priority sites, for which biological endpoints would not be necessary to decide whether open water disposal is permitted or not. Only 3 samples were considered suitable for open water disposal while 4 sediments were classified as medium-low priority sites with concentrations between the lower and the higher limit values. Although *C.volutator* registered a higher mortality percentage than *A.brevicornis* for sample CA1, considered a negative toxicity control (Table 2), *A.brevicornis* reported higher mortality values than *C.volutator* for the other two Category I sediments, specially for the fine sediment BI3, that was considered toxic for this last species. The possibility of false positives due to little sediment tolerance is a key issue when selecting a test organism for sediment toxicity assessment. Moreover these two amphipod species have different habitat preferences: *Corophium volutator* prefers muddy sediments while *Ampelisca brevicornis* is a typical amphipod of fine or muddy sand mixed with shell. A reference value of 90% mud has been established for *Corophium volutator* to ensure no interference of sediment grain size in the 10-d toxicity test results (Stronkhorst et al., 2003). This value was failed by 5 sediments and 4 of these samples caused toxic effects to *C.volutator*. Other reference values are available for *Rhepoxynius abronius*, with a 15% decrease in survival when the percentage of silt-clay is higher than 80% (Tay et al., 1998). No information regarding sediment tolerance is available for *Ampelisca brevicornis* and even if it prefers sandy sediments the incidence of mortality for sediments with higher percentage of fines was similar to that obtained for *C.volutator*. In addition the correlation analysis (Table 4) identified similar correlations for both amphipod species and grain size (although none significant) though the little number of fine non-contaminated sediments did not allow to study the effect of this variable alone in the correlation analysis.

Table 4

Spearman Rank Correlations between the set of variables obtained for the 22 samples.

	Spearman rho	
Variables	***Corophium volutator***	***Ampelisca brevicornis***
% coarse	0.014	-0.057
% sand	-0.338	-0.180
% fines	0.354	0.192
% TOC	0.447	0.137
Metallic compounds		
As	0.631**	0.834**
Cd	0.542**	0.446*
Cr	0.221	0.175
Cu	0.663**	0.770**
Hg	0.556**	0.676**
Ni	0.532*	0.355
Pb	0.581**	0.741**
Zn	0.627**	0.799**
Organic compounds		
PCBs	0.400	0.392
PAHs	0.362	0.207
Ampelica brevicornis	0.680**	1.000

The sediments considered medium-low priority sites according to the CEDEX (1994) caused higher mortalities and a higher incidence of toxicity when using *C.volutator*. This can be partly explained because of the lower toxicity to *A.brevicornis* for samples named CA#, that were actually sampled in the area where the individuals used in the test were collected. These results can indicate the advantage of autochthon species by giving additional information on potential affected species and can also improve the characterization of medium-low contaminated sediments since autochthon species could have developed tolerance to different non-chemical variables of the sediment.

Both amphipod species reported a similar percentage of toxic samples (75% for *A.brevicornis* and 68% for *C.volutator*), also similar to the percentage of samples considered not suitable for open water disposal when using the number of AL exceeded (68%). The species *Rhepoxynius abronius* reported 78% incidence of toxicity for 341 sediments from California, 45% failing at least one chemical ERM guideline (Anderson et al., 1998) while *C.colo* reported a 40% of toxic samples. This difference in the sensitivity of this Australian species evidences the inadequate knowledge of species sensitivities to contaminants and how it can influence decision-making. Further studies identified *C.colo* as the less sensitive among eight different species in water-only exposures and spiked sediments with Cu and Zn (King et al., 2005), and recommended epibenthic amphipods to be more sensitive than

infaunal tube-dwellers. In this sense *C.volutator* and *A.brevicornis* are infaunal tube-dwellers although reported higher sensitivities than *C.colo* and more in the range of the recommended Australian species.

To evaluate the sensitivity to the tested species according to the chemical categories the results were compared to those reported for *C.colo* (McCreay et al., 2005), *Rhepoxynius abronius* and *Ampelisca abdita*, used to establish and validate sediment quality guidelines (Long and MacDonald, 1998a;b). Because a different number of contaminants of concern were analysed (Spain only considers 8 metallic compounds and the sum of 7 PCBs congeners while the US includes 66 analytes to be analyzed and for which available SQGs have been developed) the ERL/ERM categories have been pooled to obtain the same priority categories. A linear relationship was observed between the proportion of AL2 exceeded and the mean quotients but a higher exposure-response relationship was established when the mean quotients were used to classify the sediments. While *C.colo* did not find toxicity for sediments with mean quotients lower than 0.1, 12% of these sediments were toxic for *R.abronius* and *A.abdita* that is similar to the 16.7% reported by *A.brevicornis* in this study. *C.volutator* reported higher toxicity for this low priority sites (25%) and higher standard deviation than *A.brevicornis* , both factors possibly influenced by the handling and shipping of test organisms prior to test. When testing medium-low contaminated sediments (mean quotient between 0.11 and 0.5) both species showed similar results and similar to that reported for the U.S. sediments (30% approximately) while medium-high contaminated sediments (mean quotients between 0.51 and 1.5) reported an incidence of toxicity of around 60%, higher than the 46% reported in the U.S. although the chemical load of our sediments could be underestimated due to the little number of compounds for which action levels have been established.

The amphipod *Ampelisca brevicornis* has been successfully used for sediment toxicity assessment (Riba et al., 2004) and reported a similar sensitivity to *Corophium volutator* to metal rich natural sediments (Riba et al., 2003). Even if the total number of samples limits to make sound conclusions it seems that both species can be used for dredged material toxicity. Some differences were reported when testing low and medium-low priority sediments for which interfering factors can influence critically in the final classification of the samples. Part of these differences could be explained by the lower sensitivity of *A.brevicornis* to some sediment sampled in the same area where the individuals were collected although further studies should be directed to clarify the possible influence of handling and shipping on test organism sensitivity and performance. A wide number of

amphipod species have been used already to evaluate contaminated sediments and dredged material although test species selection is still uncertain. The SETAC (1993) recommended preferably *Corophium volutator* but considers other locally available amphipods, particularly where routine, standard test methodologies already exist. *Corophium volutator* is the recommended test species in different countries and has been widely used in Europe (Stronkhorst, 2003; Pellegrini et al., 1999) but other species such as *Gammarus locusta* has been successfully evaluated as an alternative test where the existing tests cannot be applied (Costa et al., 1998). Previous studies are already available for *Ampelisca araucana* (Larrain et al., 1998), *Bathyporeia sarsi* (Van den Hurk et al., 1997); *Mycrodeutopus gryllotalpa* (Cesar et al., 2002) and for other *Corophium* sp. (Onorati et al., 1998). The results of this study suggest the amphipod *Ampelisca brevicornis* as suitable test species for dredged material toxicity assessment when *Corophium volutator* is not available. This species reported similar sensitivity to other amphipods traditionally used for contaminated sediment and dredged material characterization and offer certain advantages to other non indigenous amphipods apart from being ready available due to higher sediment tolerance and higher precision.

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Ecotoxicological Characterisation of Sediments from Spanish Harbours[1]

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Abstract

This paper presents the toxicological responses of three species upon exposure to surficial sediment samples from Spanish harbours. The bioassays performed included three different test species and five endpoints: the Microtox® Solid Phase test, the test using the irregular sea urchin *Echinocardium cordatum* and the clam *Ruditapes philippinarum*. The endpoints considered included mortality after 14 days and the burrowing activity measured as non-buried organisms after 48 hours of exposure to the whole sediments. In addition the results were compared with the results of the physico-chemical characterisation, which included the determination of the metals Cd, Cr, Cu, Hg, Ni, Pb and Zn, the metalloid As, certain polycyclic aromatic hydrocarbons and polychlorinated biphenyls, and other non-chemical sediment properties (grain size and total organic matter). The chemical characterisation identified more than 60% of the samples as not suitable for open water disposal according to the high chemical concentrations. However, the bioassays determined different degrees of toxicity depending on the test species and the endpoint considered. The Microtox® SPT was the most sensitive bioassay with the highest incidence of toxicity for low, medium and contaminated sediments. The irregular sea urchin showed a higher sensitivity to sediment contamination than clams as toxic effects on bivalves started to appear at concentrations higher than the upper limit values for dredged material disposal. These results indicate the suitability of the Microtox® SP test and the bioassay using sea urchins in a sediment toxicity test battery for dredged material characterisation and management, being these toxicological responses correlated with the contamination present in the sediments.

Keywords: Microtox®, *Echinocardium cordatum, Ruditapes philippinarum,* sediment toxicity test battery, dredged material, solid-phase test

[1] Ecotoxicology (Enviado)

1. Introduction

In the last decades different national and international agencies have encourage the use of toxicity bioassays to characterise and manage contaminated sediments and dredged materials as they offer unique information to address potential toxic effects caused by all the chemical species present and at their actual bioavailable concentrations. The number and type of tests needed to provide a weight-of-evidence for assessment of possible biological effects will depend on the questions being addressed and the confidence needed for decision-making, although generally two to four bioassays using several test organisms of different taxa and exposure pathways are recommended (OSPARCOM, 1998). In addition, indigenous species are preferred together with standard protocols, nonetheless there are still some difficulties to identify suitable test species and test protocols on regional scales. Despite different bioassays have been standardised to different degrees (Nendza, 2002; DelValls et al., 2003) there is not still a common approach for contaminated sediment and dredged material characterisation in Europe (den Besten et al., 2003). The present study was part of a larger toxicological study of dredged sediments from Spanish harbours, the objective of which was to assess the sensitivity of different sediment toxicity tests and its utility in a test battery. This paper present the toxicological responses of the benthic sea urchin *Echinocardium cordatum,* the clam *Ruditapes philippinarum* and the bacterium *Vibrio fischeri* (Microtox®) upon exposure to whole sediment samples.

The irregular sea urchin *Echinocardium cordatum* is a common infaunal species with an almost cosmopolitan distribution found in open coasts, offshore seabed and enclosed, sheltered coasts. *E.cordatum* lives in a permanent burrow buried about 8-15 cm deep in sandy sediments, including fine, muddy and clean sand but also sandy mud. This species has been recommended by the OSPAR commission as sediment reworker ring-test and has been used for sediment toxicity assessment in the North Sea (Bowmer, 1993; Daan et al., 1990) and to test specific contaminants (Stronkhorst et al., 1999; Daan & Mulder, 1996). Later on the protocol was standardised for contaminated sediments and dredged material (RIKZ, 1999) and was applied in the Netherlands in a sediment toxicity test battery (Stronkhorst et al., 2003).

The bioassay using juvenile bivalves is not standardised but bivalve molluscs have been identified as suitable indicators of sediment contamination, especially metals, and it fulfils many of the criteria set down for species selection in toxicity testing: it is available all year round because it is a commercial species, it is easy to maintain in the laboratory,

economically relevant and has a wide geographical distribution. Different species have been used such as *Scrobicularia plana* (Byrne and O'Halloran, 1999; Riba et al., 2004a; 2003), *Macoma balthica* (Bryan et al., 1985; Duquesne et al., 2004), *Tapes decussatus* (Mariño-Balsa et al., 2003), or *Mya arenaria* (Phelps, 1990) but we selected the Manila clam, *Tapes semidecussatus* or *Ruditapes philippinarum* as it is also known, a soft sediment dwelling mollusk that has been previously used for sediment toxicity assessment (DelValls et al., 2003; Riba et al., 2004b).

To complete the test battery the commercial Microtox® SP test have been included, which evaluates the toxic effects on the metabolic pathways of the marine bacteria *Vibrio fischeri* exposed to whole sediments. Different microbial tests have been used on environmental toxicity assessments and there are already some commercial devices that are fully standardized. These tests are easy to handle and are needed of shorter testing times than other tests that consider more complex biochemical functions in bacteria and higher organisms (Munkittrick et al., 1991; Mowat & Bundy, 2001). The Microtox® SP test has been used to screen for sediment acute toxicity in different surveys (Stronkhorst et al., 2003; Pellegrini et al., 1998) and has been recommended as part of the characterisation assessments for contaminated sediments and dredged material management in different national programs (den Besten et al., 2003). Furthermore the reproducibility of results among different laboratories have been previously assessed in Spain, suggesting that it may be a suitable methodology for dredged material characterisation (Casado-Martínez et al., 2006a). The results of the chemical and ecotoxicological assessments were linked to evaluate the use of these bioassays as part of the general framework for dredged material management in Spain.

2. Materials and Methods

2.1. Environmental samples.

Sediment samples were collected at five different Ports around the Spanish coasts with a 0.025 m^2 Van Veen grab from approximately the top 20 cm of the sediment. Samples were brought to the laboratory and subsampled for the physico-chemical characterisation. Sediments were stored at 4ºC less than two weeks prior to tests.

The physico-chemical measurements followed the general recommendations for dredged material. Analytical methods were described in detail elsewhere (Casado-Martínez et al., 2006a; 2006b). Grain size determinations followed UNE 103 101 and total organic

matter content (TOM) was measured by loss of ignition at 550ºC and gravimetric determination as recommended for small dredged volumes. For the rest of measurements sediments were dried at 40ºC for 24 hours. Metals were determined in microwave acid-digested samples in Teflon vessels. Hg was determined by cold vapour atomic absorption technique and As by hydride generation, and both quantified using atomic absorption spectrometry. The concentrations of Cd, Pb, Cu, Zn and Cr were determined using flame or furnace atomic absorption spectrometry, depending on the metal content. Polychlorinated biphenyls and polycyclic aromatic hydrocarbons were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment and concentration and clean-up with column chromatography. Determination of PCBs was made with gas chromatography with electron capture detection (GC-ECD) (EPA 8080) and PAHs were determined with HPLC with fluorescence detection (EPA 8310).

2.2. Toxicity tests

2.2.1. Bioassay using luminescent bacteria: Microtox® SP test

The bioluminescent bacterial test uses a standardized culture of a selected strain of a marine bacterium, *Vibrio fischeri NRRL B-11177* (formerly referred to as *Photobacterium phosphoreum*). Light is emitted as result of a metabolic pathway linked to the cellular respiration, so disruption of normal cellular respiration results in a rapid decrease in light emission. The test followed the SP test (AZUR, 1998), which measures the light emission of the bacteria that are recovered by filtration after incubation in direct contact with a slurry of whole sediment and diluent. Test parameters and conditions to develop the test in the laboratory have been previously reported by the authors (Casado-Martínez et al., 2006c) and are summarized in table 1.

2.2.2. Bioassay using Ruditapes philippinarum

Clams were obtained from a commercial hatchery and were acclimated to laboratory conditions in clean sea water for at least two weeks before the test was started. During this period animals were fed a mixture of different species of micro algae (*Tetraselmis chuii, Isochrysis galvana* and *Chaetoceros gracilis)* and were maintained in open water system. Test parameters and conditions have been previously reported by the authors (Casado-Martínez et al., 2006d) and are summarized in table 2. Briefly sediments were added to the test chambers to have approximately a 5 cm layer and clean sea water in a relationship 1:3. Test chambers were aerated at least 12 hours in advance to the addition of the organisms and

then 40 organisms were randomly selected and added to each replicate. The sub-lethal test was finished after 48 hours of exposure by counting the number of not-buried organisms. After 14 days of exposure the number of surviving organisms was assessed by sieving the sediment through a 0.5 mm mesh and the percentage of mortality was derived. Samples were considered toxic if the results were statistically different ($p<0.05$) from a negative toxicity control.

2.2.3. Bioassay using Echinodardium cordatum

Individuals were collected from a clean area in Cádiz with the help of a diver and when a large number of them were collected they were brought in coolers to the laboratory in clean oxygenated sea water. Animals were acclimating to test conditions for one week at 15±2ºC with running sea water and a sediment bottom layer of 10 cm. No additional food was given during this period.

Test set-up followed the standard Dutch regulation (RIKZ, 1999). Test parameters and conditions for the test are summarized in table 3. Test was conducted in 12 L beakers with overflow ports at 9 cm. Test chambers were filled with sediment to a level of 6 cm and allowed to stand for one hour to let the sediment settle. After this, the water pump was adjusted to a flow rate of 10±2 L per 24 hours and, once the aeration was adjusted such that air bubbles could be observed, the exposure system was allowed for 24 hours to ensure that it was working properly. Even if a large number of organisms were collected to conduct the bioassay following the standard operating procedure, the high mortality during shipping and acclimation did not allow using more than 12 animals per sample. Nonetheless the bioassay was conducted for logistical reasons. On the first day salinity, pH, temperature and dissolved oxygen concentrations were measured. Then organisms were weighted (5-10 g) and counted to form groups in the same number as test chambers and they were placed at regular intervals on each one. After 48 hours the number of buried organisms was assessed, considering an individual as completely buried when the upper side of the organism was level with the sediment surface. During the 14 days of exposure the organisms and the exposure system were checked daily and, at the end of exposure, animals were counted to determine the number of surviving organisms.

2.3. Data analysis

The results of the Microtox® assay were expressed as an IC50 value on dry weight basis and no further correction was applied as recommended in EC (2002). The toxicity

guideline applied by this environmental agency says that any test sediment from a particular sampling station and depth is judged to have failed this toxicity test if its IC50 is <1000 mg/L, regardless of grain size characteristics. Nonetheless it includes a second guideline for sediments that are comprised of <20% fines and has an IC50≥1000 mg/L. In this case the result must be compared against a sample of clean reference sediment with a percent of fines content that does not differ in more than 30% from that of the test sediment.

The toxicity guideline to interpret the results of the bioassay using *E.cordatum* and *R.philippinarum* was the statistical difference from a control sediment. Due to the little number of cases for each sample and the low response percentages (<5%), parametric statistical analyses could not be used. Thus the Fisher test to determine for which samples the mortality response was significantly different (p=0.05) to that registered for the negative toxicity control, in this study a clean sand (sample CA1). These analyses were developed using the program 'Simple Interactive Statistical Analysis' (SISA), available at http://home.clara.net/sisa/. The Spearman Rank correlation analysis was used to find significant relationships (at p<0.01 and 0.05) between the physico-chemical results and the toxicity endpoints. This analysis was developed by means of the statistical program STATISTICA 5.0®.

Table 1

Test parameters and conditions followed for the Microtox® SPT using the marine bacteria *Vibrio fischeri* (from Casado-Martínez et al., 2006c).

Facilities and equipment	Photometer (Microtox® Model 500 Analyser) reading light output at 490±100 nm; incubator for single cuvette at 5.5±1ºC; controlled-temperature room or incubator at 15±0.5ºC.
Reconstitution Solution	Pure, non-toxic water
Control/dilution water	Diluent purchased from the same commercial supplier or 3.5% NaCl solution
Test temperature	15±0.5ºC
Sample pH, salinity and color/turbidity	No adjustment or correction
Aeration	Not required
Sub samples for moisture content	3 replicates of 5±0.2 g dried at 100±0.5ºC for 24 h.
Primary dilution	7.00±0.05 g whole, homogenized sediment in 35 mL dilution water in a beaker, mixed for 10 min on a magnetic stirrer, at a rate such that the vortex depth is half the height of the liquid level.
Test concentrations	Maximum test concentrations normally 197000 mg/L (19.7%, wet wt:vol) on wet-weight basis with two-fold dilutions, for a total of 12 test concentrations in disposable polystyrene tubes; four control solutions; left for ten minutes to equilibrate to the test temperature
Test species	*Vibrio fischeri* strain NRRL B-11177, reconstituted by swirling vial three to four times, and mixed 10 times with 0.5 mL pipette and held at 5.5±1ºC for 30 minutes.
Inoculum	20 µL into each test concentration, mixed three times with 1.5 mL pipette.
Incubation	20 min at test temperature, filter columns inserted into top of SPT tubes above surface of test concentrations
Filtrate transfer	500 µL into disposable glass cuvettes at test temperature
Observations	Light levels of all test filtrates and controls measured
Endpoint	IC50 (mg/L), calculated by software; normalized for moisture content of sediment
Reference toxicant	Performed within one month of each test, using suitable positive control sediment and the procedures and conditions for measuring the toxicity of test sediment

Table 2

Test parameters and conditions followed for the test using juvenile bivalves in the laboratory (from Casado-Martínez et al., 2006d).

Parameters	**Conditions**
Test type	Static. On whole sediment.
Temperature	15-20ºC (19ºC recommended)
Salinity	36-40
Photoperiod	Natural of the season. Also continuous light.
Test chambers	Glass, 10-15L, (type aquaria recommended).
Volume of sediment	1.5-2.0 L (1:3 sediment/water)
Volume overlying water	6-8 L (1:3 sediment/water)
Water renewal	Not necessary
Size and state of organisms	*Ruditapes philippinarum* 1-2 cm de diameter.
Number of organisms per chamber	20
Number of replicates	2
Feeding regime	No
Aeration	12 h before introducing the organisms. To ensure dissolved oxygen concentrations equal or higher than 90% of saturation
Overlying water	Clean sea water. Also artificial.
Water quality	Daily measurements of temperature. pH, ammonia, salinity and dissolved oxygen, at least in the beginning and at the end of the test.
Test duration	Lethal 14 days. Sub-lethal 48 hours.
Endpoints	Survival
Test acceptability	90% survival in the negative toxicity control.

Table 3

Test parameters and conditions followed for the test using *Echinocardium cordatum* in the laboratory (Adapted from RIKZ, 1999).

Parameters	**Conditions**
Type of test	Flow through system. On whole sediment.
Temperature	18 ºC
Salinity	35±2
Photoperiod	Natural of the season.
Test chambers	Glass, 15×50×14 cm with flow at 8-9 cm
Volume of sediment	4.5 L
Volume of overlying water	Test chambers are filled till it flows.
Water renewal	Pump adjusted to 10±2 L per 24 hours
Size of test organisms	5 g
Number of test organisms per chamber	6
Number of replicates	2
Food regime	No
Aeration	To ensure dissolved oxygen concentrations higher than 60 % saturation.
Overlying water	Clean sea water.
Water quality measurements	On days 0, 3, 6, 9, 12, 14 and if any mortality: salinity, pH, temperature and dissolved oxygen.
Test duration	14 days
Endpoints	Survival and burrowed organisms after 48 hours.
Test acceptability	Survival in control higher than 90%

3. Results

3.1. Sediment physico-chemical characterisation

The results of the chemical analyses of organic and inorganic contaminants and the non-chemical parameters of the sediment are further discussed in Casado-Martínez et al. (2006b). The sediments tested comprised the three categories established in the Recommendations for Dredged Material Management used in Spain (CEDEX, 1994). The lower limit values (Action Level 1) for each single compound were used to identify low priority sediments and the higher limit values (Action Level 2) to identify whether sediments were potentially toxic due to the high chemical content and hence not suitable for open water disposal as recommended for dredged materials in Spain (Table 4). The sediments falling into Category I reported very different sediment properties, namely grain size distribution and organic matter content. Sample CA1 was a typical sand with a low percentage of fines and organic matter content, sample H4 was coarser and BI3 reported more than 90% fines and organic matter content higher than 15%.

The rest of sediments showed very variable percentages of fines (ranging between 31 and 99%) and organic matter content (between 3 and 24%). The sediments were also contaminated with a mixture of organic and inorganic contaminants. The sediments that fell into Category II CA3 and CA4 showed intermediate Cd concentrations while B1 and B3 reported intermediate concentrations of Hg and PCBs and for B3 also Cu. Similarly the dredged materials that fell into Category III contained multiple contaminants typical of urbanised/industrialised areas (Casado-Martínez et al., 2006b), including the entire list of priority substances (CEDEX, 1994) except the metal Cr.

3.2. Ecotoxicological assessments

3.2.1. Toxicity results

The results of the sediment toxicity assessments are summarised in Table 4. A full range of toxicity results was recorded for each test. The IC50 values registered by the Microtox® device ranged from 110 to 29028 mg/L d.w., which is far below and far above the toxicity criteria of 1000 mg/L d.w. and corresponded to a contaminated mud and a clear sand, respectively (Casado-Martínez et al., 2006a).

This clear sand was considered the control sediment to which compare the results of mortality and burrowing activity on clams and sea urchin upon exposure to the rest of treatment dredged materials. As expected this sample showed results on the lower range for all sediments, with 1 to 8% of toxicity and always considered not toxic, through similar percentages were reported for some sediments which fell into intermediate and high priority categories. The sea urchin mortality results ranged between 8 to 92%, which is similar to the range of results on the sea urchin burrowing behaviour (8-83%). Similarly samples were considered toxic when mortality/not buried organisms exceeded 50%, which corresponds to 42% when results are corrected for the negative toxicity control.

On the contrary the lethal and sublethal toxic responses on clams upon exposure to the sediment samples showed important differences as clam mortality ranged from 0 to 66% while the percentage of clams non-burrowed after 48 hours obtained for the sediments tested ranged from 0 to 15%.

Table 4

Toxicity responses upon exposure to whole dredged materials. Results are expressed as percentage except those from the Microtox® SP test, which are expressed in mg/L d.w.

	Microtox®	***E.cordatum***		***R.philippinarum***	
	SP test	**Mortality**	**Burrowing activity**	**Mortality**	**Burrowing activity**
Category I					
CA1	29028	8	8	1	3
H4	n.a.	25	17	1	5
BI3	280*	25	8	0	3
Category II					
CA3	168*	42	8	1	4
CA4	198*	67**	25	0	2
B1	2920	33	33	6	0
B3	705*	25	25	0	0
Category III					
H1	152*	83**	83**	35**	2
H2	647*	92**	67**	66**	10
H3	1225	58**	33	48**	13
CA2	110*	42	0	0	15
B2	578*	33	17	2	2
B4	358*	25	33	1	0
C1	331*	33	25	0	2
C2	743*	17	17	2	4
C3	1160	17	8	0	2
C4	567*	50**	50**	5	0
BI1	506*	58**	25	2	2
BI2	461*	67**	50**	0	2

* Means toxic according to the Canadian guideline of 1000 mg/L d.w. for dredged material.
** Means statistically different from the negative control at $p<0.05$.

3.2.2. Incidence of toxicity and comparative test sensitivity

The sensitivity of sediment toxicity tests, defined as the proportion of samples classified as toxic among the total that were tested (McCready et al., 2004), and the total incidence of toxicity was: Microtox® SP test (77%)>sea urchin survival (37%)>sea urchin burrowing activity (21%)>clam survival≈clam burrowing activity (16%) (Fig. 1).

Considering the incidence of toxicity by management categories the Microtox® SP test was the only bioassays that recorded some proportion of toxic samples in the low

priority category as this test showed very variable results for Category I materials, covering the full range of toxicity results (Fig. 1; Table 4). No IC50 value could be reported for sample H4 because it was not possible to pipette the sediment suspension needed to perform the test and the IC50 value for the uncontaminated sand was extremely high (29000 mg/L d.w.). Furthermore the fine sediment BI3 was considered toxic as the IC50 value failed the toxicity criteria 1000 mg/L d.w. considered in this study (280 mg/L d.w.). The rest of bioassays and endpoints did not identify significant toxic effects for none of these low priority sediments.

Similarly the highest incidence of toxicity for Category II materials was recorded for the Microtox® SP test -75% of these sediments were considered toxic- followed by a 25 % of samples causing a significant reduction on *E.cordatum* survival while these intermediately contaminated sediments did not cause significant toxicities on the rest of toxicity endpoints considered.

On behalf of the Category III materials, more than 80% of the samples falling into this high priority category caused toxic effects to the bacteria *V.fischeri* while for the sea urchin test the percentage of toxic samples dropped to 50% and 33% for the lethal and sublethal endpoints. The bioassay using clams was the less sensitive test, with only 25% of highly contaminated sediments reducing significantly clam survival while the burrowing activity offered similar values for the highly contaminated sediments to that reported for the uncontaminated sediments.

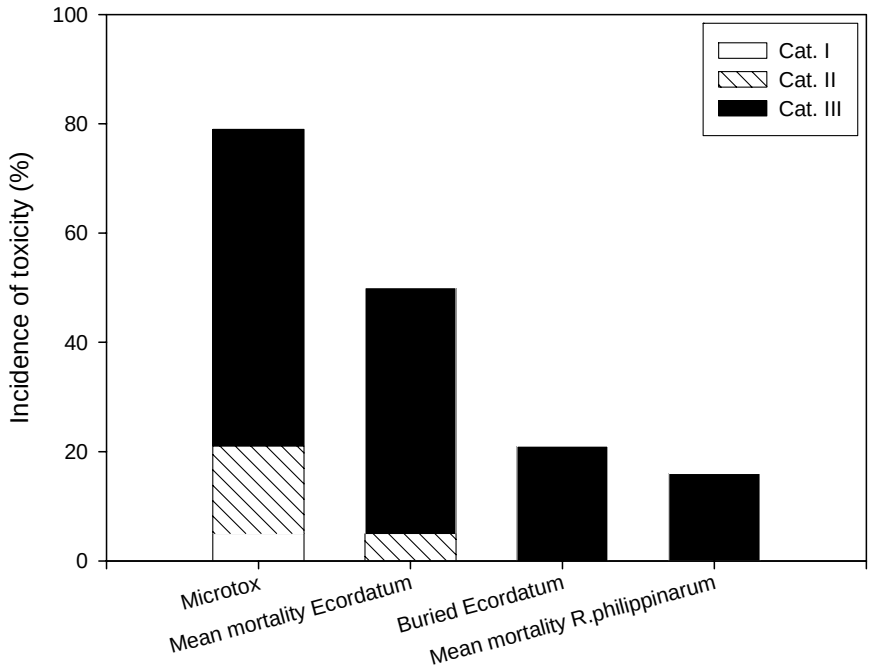

Fig. 1. Incidence of toxicity for each sediment toxicity test.

3.2.4. Relationships between toxicity and physico-chemistry of sediments

The correlation analysis identified significant relationships between the sediment properties (percentage of fines and organic matter content) and the Microtox® SP test and the sea urchin mortality at different p levels ($p<0.01$ and $p<0.05$; Table 5). Conversely these two bioassays were inversely correlated with the proportion of sands (at $p<0.01$). No significant correlation was found between the Microtox® and any single chemical concentration while the sea urchin seems to be affected by the metal As, and also Cu when considering the burrowing activity ($p<0.05$). Clam survival was correlated with the metals Cu and As (not significantly) while the burrowing activity was inversely correlated with Cr and the concentration of total PAHs ($p<0.01$ and $p<0.05$ respectively). It is worth mentioning that, despite the low correlation between the toxicity responses and the single chemical concentrations, the Microtox® SP test and the sea urchin lethal endpoint were significantly correlated with the mean quotients (at $p<0.05$), which are a measure of the sediment chemical load that integrates the number and the extent of the sediment quality guidelines exceeded (Casado-Martínez et al., 2006b).

4. Discussion

The main objective of this study was to evaluate different toxicological responses for dredged material characterisation and to investigate on the performance of each test for dredged material management. Thus the results are not discussed to investigate on the spatial distribution of toxicity, but the particular sediment properties and attributes may serve as indicators for some erroneous data. The comparative test sensitivity found by far the Microtox® SP test as the most sensitive bioassay, which is in agreement with previous studies that also found this microbial test (following different exposure phases and test protocols) as the most sensitive endpoint in a sediment toxicity test battery (Pedersen et al., 1998; McCready et al., 2004). The incidence of toxicity was 77% while the percentage of samples classified as Category III was 63%. This may indicate that some false positives may appear when testing dredged sediments (understood as sediments that are considered toxic even though the cause of toxicity cannot be directly attributed to the sediment contamination) as this test classified as toxic some sediments that fell into Category I.

Table 5

Spearman rank correlation analysis results as Spearman *R* for each pair of variables included.

Variables	**Microtox®**	***Ecordatum***		***R.philippinarum***	
	SP test	**Mortality**	**Burrowing activity**	**Mortality**	**Burrowing activity**
Non-chemical					
% gravel	0.053	-0.348	0.002	-0.142	-0.306
% sand	0.723**	-0.577**	-0.253	0.158	-0.158
% fines	-0.526*	0.582**	0.196	-0.080	0.034
% TOC	-0.574*	0.518*	0.167	-0.193	-0.031
Metals					
As	0.060	0.500*	0.541*	0.311	0.094
Cd	0.102	0.167	0.342	0.161	-0.197
Cr	0.228	-0.102	0.336	0.102	-0.651**
Cu	0.109	0.382	0.539*	0.350	0.021
Hg	0.156	0.023	0.309	0.082	-0.198
Ni	-0.110	0.423	0.455	0.024	-0.343
Pb	0.197	0.056	0.396	0.166	-0.230
Zn	0.196	0.194	0.425	0.269	-0.099
Organic micropollutants					
PCBs	0.050	-0.160	0.048	-0.185	-0.309
PAHs	0.166	-0.172	0.173	-0.194	-0.563*
Toxicity bioassays					
Microtox®					
E.cordatum					
Mortality	-0.310				
Burrowing activity	0.100	0.639**			
R.philippinarum					
Mortality	0.322	0.358	0.545*		
Burrowing activity	-0.194	0.083	-0.392	0.099	
Mean-AL2-q	-0.574*	0.518*	0.167	-0.193	-0.031

*at $p<0.05$
**at $p<0.01$

Nonetheless it is difficult to discern whether these toxic effects are caused by other contaminants not measured during the physico-chemical characterisation as only a limited number of all the contaminants that may be present in harbour sediments are actually measured. If the test is used as an screening method further assessments would investigate on this possibility although they are of major importance if results are used for decision making. In this case it may be helpful to investigate if other sediment attribute such as grain size, organic matter, or other nuisance variable such as hydrogen sulphide or ammonia contribute causing part of the toxic response registered. As the data retrieved from the physico-chemical characterisation is limited it is difficult to ensure when we are really facing

false positives. Nonetheless the correlation analysis identified significant relationships between the non-chemical variables and the IC50 values, indicating that low toxicities are usually related to sands and coarse sediments while muddy, rich in organic matter dredged materials are related to high toxicities (Table 5). This is in agreement with previous studies that stressed the importance of sediment texture as the major confounding factor for interpreting the Microtox® SP test results (Stronkhorst et al., 2003; Ringwood et al., 1997) and may help explaining the toxic effects recorded for low priority dredged materials.

In the case of sandy and coarse sediments the problem lays on the difficulties to pipette properly the sample thus to elaborate the correct dilutions used to calculate the IC50 value. This may be easily seen with a thorough study of the light inhibition curve. In this case the Canadian guidelines for the disposal at sea program recommend to perform reference sediment of similar properties but absent of contamination in parallel with the treatment sediment to which compare the IC50 value (EC, 2002). Nonetheless, sediments with a high proportion of fines are not exempted from some controversy as light emission can decrease critically due to poor recoveries of the bacteria after sample filtration and dredged materials from Spanish ports are principally mud with a high proportion of organic matter content (Casado-Martínez et al. 2006b). Ringwood et al. (1997) published the first interpretation guidelines for this test as ranges of IC50 values depending on the proportion of silt and clay in the sample, and later on Stronkhorst (2003) developed an algorithm using data from previous studies. The application of this correction to silty sediments from Dutch harbours showed that the physical interaction of the fine sediment fraction was the sole cause of light inhibition in 33% of 257 sediments and 60% of 22 coastal reference sediments tested (Stronkhorst et al., 2003), but the Dutch sediments had no more than 74% fines while our sediment ranged from 1 to 99% of fines. This may explain that the new data generated after correcting our results were more difficult to interpret than the uncorrected values (data not shown) although as Ringwood et al. (1997) suggested unless sediment composition is taken into consideration, the number of false positives may be high, i.e., all sites with silt-clay concentrations >20 to 25% will be classified as toxic, which is in agreement with our results.

The toxicity criteria may also determine an extremely high incidence of toxicity, but we did not consider the use of the statistical difference as it is usually difficult to find a sediment with similar properties to the sediment being tested but absent of contamination, especially sediments with a high proportion of fines. The use of the Canadian criteria (1000 mg/L on dry weight basis) identified a similar incidence of toxicity to previous studies on

dredged materials. A large database of Dutch harbour sediments reported a similar incidence of toxicity after the correction for the fine fraction and using a limit value for toxicity of 48 Toxic Units (Stronkhorst et al., 2003) while Porebski et al. (1999) found all sediments toxic when evaluating the Microtox® SP test for dredged sediment assessment and considering marginally toxic the IC50 values between 1000 and 5000 mg/L d.w. but still this bioassay is considered for dredged material toxicity assessment. It is because, in general, microbial toxicity tests are much easier to perform than toxicity tests with benthic invertebrates, they are relatively cheap and useful for screening purposes even if, as the experimental set up is similar to single species toxicity tests with benthic invertebrates, therefore they may suffer from the same drawbacks (van Beelen, 2003). In this same line a maximum 90% fines reference value has been also reported for *E.cordatum* (Postma et al., 2002) and this value was failed for some of the sediments that caused significant effects on the sea urchin mortality test, nonetheless toxicity was only reported for medium and high contaminated sediments and not for the low contaminated ones. The lack of toxicity for Category I sediments evidence the absence of false positives when using the sea urchin test, yet the use of the mean quotients identify some anomalous toxicity for fine grained sediments when comparing the results with the standard 10 days amphipod lethal test, a more sensitive endpoint than this other benthic organism (Casado-Martínez et al., accepted).

As for the Microtox® SP test, our study reported a similar incidence of toxicity when testing dredged materials with the sea urchin mortality test to that reported previously for Dutch sediments, with 50% of sediments considered toxic (Stronkhorst et al., 2003). On the contrary, there is not a database to which compare our results on the clam mortality test. The bioassay using juveniles of *R.philippinarum* was not sensitive enough to detect toxicity at medium contaminated sediments as the chemical concentrations at which toxic effects started to appear were higher than the maximum guidelines used to consider materials not suitable for open water disposal. These results negates the usefulness of this test for dredged material characterisation and management in Spain as ecotoxicological assessments are considered for sediments falling into Category II. Nevertheless the slight relationship between the sediment Cu concentration and the clam mortality and other studies indicate the suitability of this bivalve for testing sediments with known Cu contamination (Riba et al., 2003; 2004b). These previous studies also considered the burrowing activity as a suitable endpoint to investigate for sublethal effects that may lead to high predation on this species. Despite this endpoint has been previously used for sediment

toxicity assessment (Casado-Martínez et al., 2006d) our results indicate that this endpoint is not suitable for dredged material characterisation as it provides little discrimination among sites. Furthermore the significant inverse relationships between this endpoint and the PAHs and Cr concentration in sediments (at $p<0.05$ and $p<0.01$, respectively) also negates the usefulness of this endpoint for dredged materials that are not contaminated with As and Cu, which are the only contaminants that reported positive correlation coefficients.

Despite the little correlation between the sea urchin mortality and the single chemical contaminants (the sea urchin mortality was only correlated with the metalloid As) the correlation coefficients are positive and evidence some increasing toxicity when increasing the concentrations of metals in sediments although it is worth mentioning that, as for the bioassay using clams, the correlation analysis is critically influenced by the toxicity caused by the sediments H#, that reported the more conspicuous concentrations of both As and Cu (Casado-Martínez et al., 2006b). Both the sea urchin and the clam are benthic organisms that live in close association with the sediments and have similar behavioural traits, they have different feeding strategies that determine the exposition to contaminants via differing combinations of food sources, pore waters, sediment particles and overlying waters (King et al., 2005). While the sea urchin *E.cordatum* lives buried and feeds on the detritus in the sediment that actually ingests, the clam *R.philippinarum* is usually buried and is a filter organism feeding on the overlying water. In this respect these results may indicate the higher solubility of Cu in the overlying water due to different geochemical properties of the sediments that was explaining part of the clam mortality results ($p<0.05$) and the sea urchin burrowing behaviour ($p<0.05$).

The correlation analysis also served to support the hypothesis that grain size distribution and organic matter influence the Microtox® results and the sea urchin mortality. Nevertheless fine grained sediments with high organic content are usually sink for many pollutants thus these tests were also the best correlated with the mean quotient. The influence of the sediment grain size in the high incidence of toxicity should be further studied due to socio-economic constraints, especially if these results are used as pass/fail criteria for decision-making. False positives and negatives of toxicity can lead to environmental, social and economic impacts but further analyses would identify the absence of potential toxic effects. Because adequate protection is a constrain when dealing with this type of samples we recommend to use a complete battery of tests to have real data on different organisms and exposure routes, although the sea urchin bioassay seems to evaluate toxicity though both exposure routes via sediment and overlying water ingestion if

both endpoints are used thus this test could be preferred instead of the test using juvenile clams. On behalf of the Microtox® SP test, it offered satisfactory results as a screening tool and was sensitive to toxicity from almost all priority sites, nonetheless the overprotective nature of this test with respect to the clam or the sea urchin toxicity test, which seem less sensitive that the bacterium *V.fischeri* should be taken into account. From a regulatory point of view the chemical guidelines serve to predict the incidence of toxicity of the Microtox® which validates the use of this test as a screening tool. Although there are some interfering factors, principally the proportion of fines in the sediments, which increase the related false positives of toxicity, its overprotective nature could be accepted since further analyses would support the hypothesis of its ubiquity. If we consider the use of the other two bioassays, both can be used to assess the toxicity to benthic organisms but we should take into account the differences in sensitivity of the endpoints if they are to be used to support decision-making.

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Liquid versus solid phase bioassays for dredged material toxicity assessment.[1]

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Abstract

Since 1994 the results of the analyses of key chemical compounds (trace metals, polychlorinated biphenyls and polycyclic aromatic hydrocarbons) and the comparison with the corresponding sediment quality guidelines (SQGs) are used in decision-making for dredged material management in Spain. Nonetheless in the last decades a tiered testing approach is promoted for assessing the physical and chemical characteristics of dredged sediments and their potential biological effects in the environment. Bioassays have been used for sediment toxicity assessment in Spain but few or no experiences are reported on harbor sediments. We studied the incidence of toxicity in the 7d bioassay using rotifers (*Brachionus plicatilis*) and the 48h bioassay using sea urchin (*Paracentrotus lividus*) embryos over a series of experiments employing 22 different harbor sediment elutriates. The relative performance of this exposure phase was not comparable to data on the 10-d acute toxicity test using the burrowing amphipod *Corophium volutator* and the polychaete *Arenicola marina,* carried out on the whole sediments. These results evidence the importance of the exposure route and the test selected in decision-making, as the toxicity registered for the undiluted elutriates was largely due to the different solubility of sediment-bound contaminants. This work and other studies indicate that for many sediments, a complete battery of test is recommended together with physico-chemical analyses to decide whether dredged sediments are suitable for open water disposal or not.

Keywords: dredged material characterisation, test battery, *Ampelisca brevicornis*, *Corophium volutator*.

1. Introduction

Ports, rivers and water ways often need regular dredging to keep them open for navigation. Environmental concerns arise when dredged sediments are anoxic and particularly if they come from harbours or industrialized estuaries, since these sediments

[1] Environment International (Aceptado)

can be contaminated with different substances due to poor environmental policies in the past. When these dredged materials are excavated and relocated the contaminants can be transferred to the disposal grounds, where they can affect the local benthic community. Moreover during these operations sediments are oxygenated and dispersed and the contaminants may change their chemical speciation, cease to be adsorbed on to silt particles, and then enter food chains and do harm. Several countries are already applying laboratory bioassays for sediment quality assessment and/or dredged material management. One of the issues addressed by several regulatory bodies is the development of standard and sensitive methods since effects-based testing is still under development (den Besten et al., 2003; Peters et al., 2002). This study summarises the results of two different liquid phase tests for elutriate toxicity assessment: the sea-urchin embryo-larval bioassay, that is widely applied for sediment toxicity assessment including sediment elutriate and interstitial water (Beiras et al., 2001; Carr et al., 1996) and the 7-d bioassay using a population of the rotifer *Brachionus plicatilis*, previously used on sediment pore water and elutriates in Spain (DelValls et al. 1998; Riba et al., 2004a). The results are compared with standard 10-day static toxicity tests carried out on the whole sediments: the bioassay using the burrowing amphipod *Corophium volutator* (ASTM, 1991) and the bioassay using the polychaete *Arenicola marina* (Thain and Bifield, 2001). This design allows making direct intertest comparisons and, together with the physico-chemical characterisation of the sediments, to study the performance of elutriate tests for sediment toxicity assessment in the context of navigational dredging.

2. Material and methods

2.1. Sediment sampling and chemical characterization

Sediments were sampled in the ports of Huelva, Cádiz, Barcelona, Cartagena, Bilbao and Pasajes with a 0.025 m^2 Van Veen grab from approximately the top 20 cm of the sediment. Sediments were pooled until enough volume was sampled (around 40 L) and were brought to the laboratory, where they were homogenized, sieved through a 2 mm mesh to eliminate debris and stored at 4ºC, darkness and closed hermetically no longer than two weeks prior to tests. Afterwards the sediments were subsampled for sediment chemical characterization, which followed Spanish recommendations for dredged materials (CEDEX, 1994). The analyses consisted of grain size distribution, organic matter content measured as loss of ignition and the concentration of As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, the

sum of 7 polychlorinated biphenyls and 12 polycyclic aromatic hydrocarbons. All metals were quantified using flame or furnace atomic absorption spectrometry except As and Hg, measured by hydride generation and cold vapour technique respectively. PCBs were determined by gas chromatography with electron capture detection (EPA 8080) and PAHs by HPLC with fluorescence detection (EPA 8310). Detailed information of the sediment characterization has been recently reported in Casado-Martínez et al. (2006a).

2.2. Liquid phase bioassays

Sediment elutriates

Sediment elutriates were obtained using a modification of the US EPA method (1998). Sediments were homogenized and mixed with clean seawater in a proportion 1:4 v/v (sediment:water) for 30 minutes at approximately 20ºC. The mixture was left to settle overnight and then the supernatant was siphoned. The sediment elutriates were kept at 4ºC and darkness until they were used in the toxicity tests but no longer than one week. The day the tests were initiated the elutriates were transferred to the test chambers manually and they were left to reach the test temperature without additional aeration before the addition of the test organisms.

Rotifer population decay bioassay

Test parameters and conditions followed the protocol developed by DelValls et al. (1996) and are summarized in Table 1. This test evaluates the decrease in a population of the rotifer *Brachionus plicatilis* exposed to the sediment elutriates for 7 days. The test organisms were maintained for 48 hours on starving conditions prior to tests to empty the guts and the population decrease was registered throughout the test duration counting 100 organisms under an optical loupe three times a day. The number of surviving organisms was used to calculate the time needed for a decrease of 50% of the initial population under starving conditions (LT50) using a modification of the probit method (DelValls et al., 1996). A negative toxicity control was included on each batch of samples consisting on the same sea water used for culturing the test organisms and to obtain the sediment elutriates. The results were corrected for the corresponding control to compare different batch of experiments.

Sea urchin embryo-larval bioassay

Fecundation and test conditions followed the protocol developed by Fernández (2002) and is summarized in Table 1. Gametes were obtained from a single male and

female sea urchin by direct extraction with a pipette and, once the fecundation was successfully completed, embryos were introduced in 25 mL vials with the sediment elutriates at 20ºC to a density of 20-30 embryos per mL. Five replicates were used per sample and a negative toxicity control consisting of clean sea water was tested in paralel with the samples. After 48 hours at 20ºC and darkness the samples were fixed with two drops of 40% formaldehyde. The measured endpoint was embryogenesis success measured in 100 organisms per replicate after exposure to the undiluted sediment elutriates. The results are expressed as percentage of normal pluteus (defined as those with four well developed arms) normalized to the corresponding control.

Table 1

Test parameters and conditions for the test using a population of rotifers of the species *B.plicatilis* and the sea-urchin *Paracentrotus lividus* embryo-larval bioassay.

Parameter	**Rotifer population decay**	**Sea-urchin embryo-larval**
Type of test	Static. On liquid phase	Static. On liquid phase
Temperature	25ºC	20ºC
Salinity	38±2	38±2
Photoperiod	24 hours light	No
Test chambers	2 litres chambers for acclimation. 50 mL glass chambers for bioassay	25 mL glass chambers
Sample volume	50 mL	20 mL
Water renewal	None	None
State of the organisms and number per test chamber	A whole population under normal growth conditions; 200 organisms/ mL	20 - 30 embryos/mL
Replicates	3	5
Feeding regime	None	None
Aeration	None	None
Water quality	Temperature, salinity, pH and dissolved oxygen	Temperature, salinity, pH and dissolved oxygen
Test duration	7 days	48 h
Endpoints	Survival, TL50	Embryogenesis success (percentage of normal pluteus larvae)
Test acceptability	Decrease to 50% of initial population in clean sea water between 48 and 72 hours (LT_{50})	90% normal larvae in controls

2.3. Solid phase bioassays

The battery of solid phase tests included the 10-day static sediment toxicity test using the crustacean amphipod *Corophium volutator* and the 10-day static sediment toxicity test using the polychaete *Arenicola marina*. The results of these bioassays have been previously reported in Casado-Martínez et al. (submitted).

2.4. Data analysis

The non-parametric Fisher Exact test was performed on the toxicity tests results to identify significantly different responses (P=0.05) related to control. The algorithm and the method are included in the Simple Interactive Statistical Analysis (SISA) available on-line at (http://home.clara.net/sisa/). The toxicity results were interpreted in terms of management categories established by the limit values used in Spain for dredged material (Table 2): Category I for not contaminated sediments, Category II for moderate contaminated sediments and Category III for heavily contaminated sediments. Spearman rank correlations were used to link sediment contamination and toxicity. Correlation coefficients were developed using the statistical program STATISTICA® 6.0.

Table 2

Sediment quality guidelines (Action Levels) used in Spain for dredged material management (CEDEX, 1994). All values are expressed as mg·kg-1 except Σ7-PCB expressed as µg·kg-1.

Compound	AL1	AL2
As	80	200
Cd	1.0	5.0
Cr	200	1000
Cu	100	400
Hg	0.6	3.0
Ni	100	400
Pb	120	600
Zn	500	3000
Σ_7-PCB	30	100

3. Results

3.1. Sediment chemical characterization

Three sediment samples (CA1, H4 and BI3) fell within Category I, with all chemical concentrations lower than the corresponding AL1 (Table 2). Samples classified in Category II included CA3, CA4, B1 and B3 while the rest of samples fell within category III

according to the high concentrations reported for several contaminants. Sediments were affected by a mixture of compounds depending on the sources and activities at each port. The sediments from Huelva and Cartagena reported high metallic concentrations although Cartagena was also affected by high concentrations of PCBs. The ports of Bilbao and Pasajes reported high organic contamination with some metals exceeding the corresponding ALs.

3.2. Liquid phase bioassays

Rotifer population decay bioassay

The results of the sediment toxicity tests are summarised in Fig. 1. The negative toxicity controls for each batch of experiments were in the range for test acceptability (DelValls et al., 1996). This bioassay identified slight toxic effects for elutriates CA1, CA4, H1, H2, H3 and BI2, that reported LT50 values lower than the corresponding control, while the population of rotifers needed a longer time to decay when exposed to the rest of elutriates (Fig. 2). If we use the SQG developed by DelValls et al. (1996) to consider sediment toxicity (LT50s < 48 hours) none of the elutriates would be considered toxic to the population of rotifers under the described test conditions.

Sea-urchin embryo-larval bioassay

Even if the mean percentage of normal pluteus and the individual values for all replicates were in the range for test acceptability (> 90%), the embryogenesis success endpoint was a very variable endpoint among replicates for the treatment sediments. The highest toxic effects were reported after exposure to elutriates from Cádiz (CA#), H2, H3 and B2, with percentages of normal pluteus lower than 25% (Fig. 2). All the elutriates causing a percentage of abnormal pluteus higher than 25% were statistically different from controls (CA1, CA2, CA3, CA4, H2, H3 and B2) although samples H1, H4, BI2, B3 and BI3, that reported percentages lower than 25%, were also statistically different from controls. In general the elutriates that caused slight toxic effects to rotifers were also toxic to sea urchin embryos.

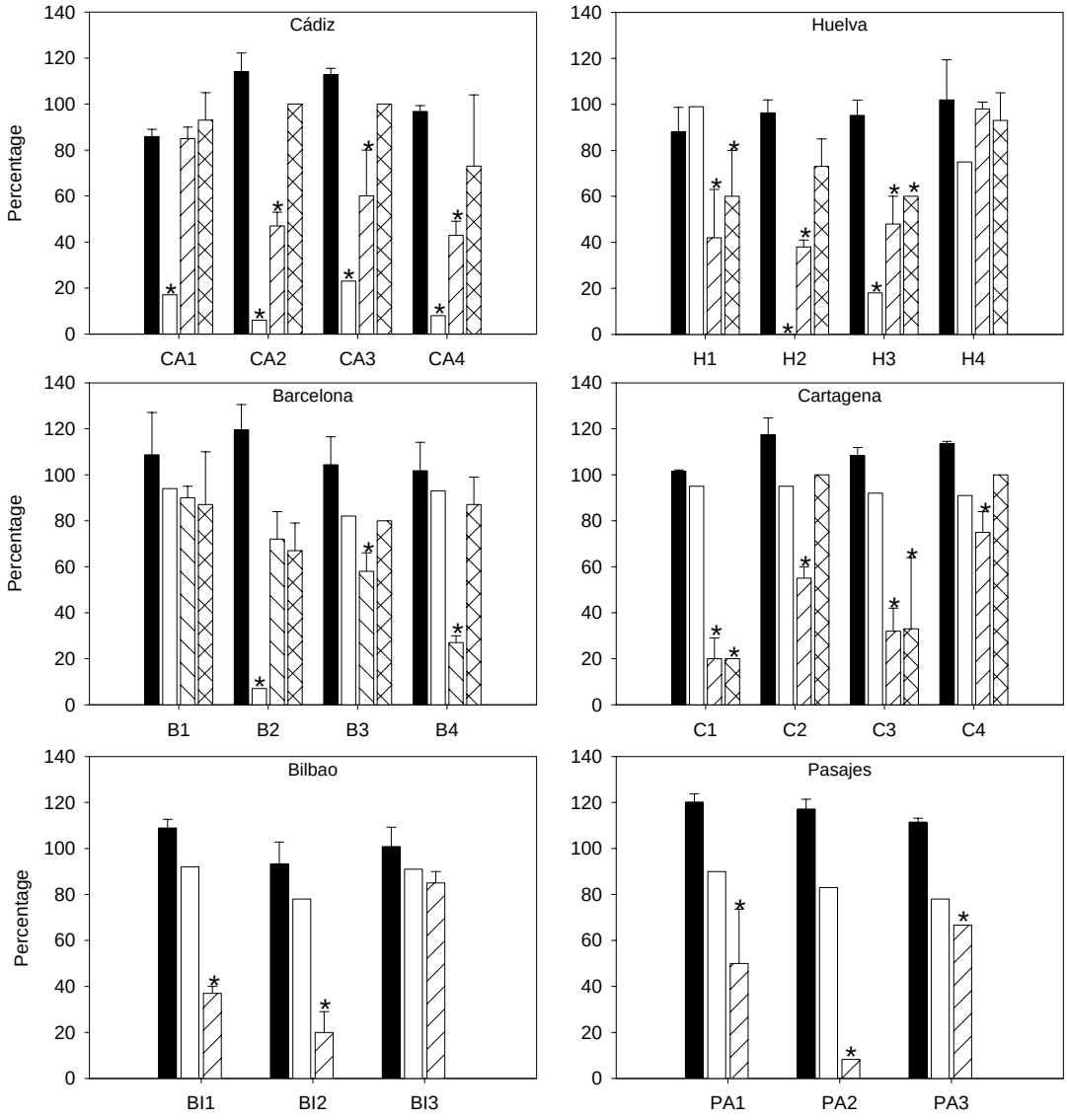

Figure 1

Results on the population rotifer decay bioassay expressed as percentage to the control response (first column); mean percentage of normally developed larvae of *Paracentrotus lividus* (second column); survival percentages of *Corophium volutator* (third column) and *Arenicola marina* (forth column). (* means significantly different from controls at $p \leq 0.05$).

3.3. Linking sediment contamination and toxicity

The biological endpoints were correlated with the chemical concentrations and other parameters included in the sediment characterisation (n=16-22; Table 3). Taking into consideration that positive coefficients would be expected (high chemical concentrations related to high toxic effects), toxicity to sea-urchin embryos was not correlated neither with the chemical compounds nor with the variables used to describe the sediment properties. The high concentrations reported in Cartagena and the absence of toxic effects may be responsible for the low correlation between contamination and this endpoint. Similarly toxicity to rotifers was not correlated neither with the concentration of metals nor with PAHs in sediments although the correlation coefficients were positive. Negative correlation coefficients, that evidence an inverse relationship between sediment contamination and toxicity, were reported between embryogenesis success and the sum of PAHs (-0.56; $p<0.01$). Rotifer population decay was inversely correlated with the proportion of fines and organic matter content (-0.29 and -0.31, respectively) but the correlation was only significant for PCBs (-0.45; $p<0.05$). On the contrary both whole sediment toxicity tests were correlated with contaminant concentrations measured in sediments (Casado-Martínez et al., submitted). Toxicity to amphipods was highly correlated with all metals analysed ($p<0.01$) except with Cr while the lethal effects in polychaetes were only correlated with As, Ni and Zn ($p<0.05$). Both solid-phase bioassays were slightly correlated with the organic contaminants (PAHs and PCBs) even though these correlations were not significant ($p<0.05$). As expected, the endpoints measured after exposure to the whole sediments were better correlated with the sediment properties. The two variables included in this study, total organic matter content and proportion of fines, are two important confounding factors for whole-sediment toxicity. On the contrary these two confounding factors seem to have a lower effect when testing sediment elutriates according to the lower correlation coefficients observed.

Table 3

Spearman rank correlation coefficients for the contaminants of concern and the liquid and solid phase bioassays. Results of bioassays were expressed so as to obtain higher toxicities for high contamination.

	Spearman rho			
	Bioassay using rotifers	**Bioassay using sea urchin embryos**	**Bioassay using amphipods**	**Bioassay using polychaetes**
% fines	-0.29	0.06	0.36	0.09
% total organic matter	-0.31	-0.12	0.46*	0.11
Metallic compounds				
As	0.08	-0.16	0.63**	0.56*
Cd	0.24	-0.23	0.54**	0.35
Cr	0.01	-0.53	0.22	0.22
Cu	0.21	0.25	0.65**	0.35
Hg	0.04	-0.18	0.55**	0.22
Ni	-0.07	-0.29	0.55**	0.57*
Pb	0.02	-0.29	0.58**	0.37
Zn	0.08	-0.19	0.62**	0.47*
Organic compounds				
PCBs	-0.45*	-0.27	0.41	0.04
PAHs	-0.19	-0.59**	0.38	0.25
Bioassays				
Rotifer population decay		-0.03	-0.17	0.13
Embryo-larval bioassay			-0.05	-0.12
Amphipod				0.65**

* = significant at 0.05; ** = significant at 0.01.

4. Discussion

Two different tests have been used to evaluate dredged material toxicity through exposure to undiluted sediment elutriates: the bioassay using a population of the rotifer *Brachionus plicatilis* and the bioassay using sea urchin embryos. Both test organisms are pelagic and would be exposed to contamination throughout the water column. Nonetheless substantial differences have been reported by these two measurement endpoints. Our results showed no toxicity or slight toxicity to the population of rotifers after exposure to the elutriates and even for 72% of the total number of samples the LT50 values were higher than that reported for the negative control. The dredged sediments tested fell within the three management categories (CEDEX, 1994), with different chemical compounds failing the corresponding AL2s -defined by the London Convention as the numeric value

for chemical concentrations above which open water disposal is prohibited (IMO, 1998)-. Embryogenesis success was a more sensitive endpoint than rotifer population decay, nonetheless the highest toxic effects were reported for the lower sediment chemical concentrations while the elutriates corresponding to the port of Cartagena, highly contaminated with metallic and organic contaminants, did not cause significant toxicities. The only contaminant of concern identified in sediments considered into category II (CA3 and CA4) was Cd, which in turn has reported lower toxicity to sea urchin embryos than other metals (Fernández and Beiras, 2001; Radenac et al., 2001). The high toxicity caused by sample CA1, that fell into category I, suggests that other toxicant not measured may contribute in elutriate toxicity. The high toxic effects could be related to the presence of hydrogen sulfide according to previous concentrations reported in the area (unpublished data). This substance, which is a natural component of highly reduced sediments, greatly increases during sediment storage under anoxic conditions (Lapota et al., 2000) and results in a very toxic effect on the sea urchin embryos (Knezovich et al., 1996). Moreover toxicity to sea urchin embryos was reduced considerably after a brief aeration of the sediment elutriates from site CA1 (Casado-Martínez et al., 2006b), which supports this hypothesis. Ammonia is another naturally-occuring toxicant that may be produced in sediments extremely rich in organic matter and results in high toxicities to different species of rotifers (Lahr et al. 2003) and sea-urchin embryos (Losso et al., 2004a; b) but no measurements were included in this study.

Further studies on chemical concentrations in the sediment elutriates would address this misfit between contamination and toxicity and would improve significantly the results of elutriate tests on behalf of dredged material management strategies. The results of the correlation study are specially interesting for the organic micropollutants, that reported an inverse cause-effect relationship with the elutriate toxicity tests. This inverse relationship was especially significant for the sea urchin embryo-larval bioassay and PAHs ($p<0.01$) and between the rotifer population decay and PCBs ($p<0.05$). Low elutriate:bulk concentrations ratios have been reported in the literature (Thompson et al., 1999) and there are numerous results on harbour elutriates showing absence of metal release from sediments (McDonald, 2005; Pedersen et al., 1998). Even positive effects on invertebrate larval metamorphosis at low metal concentrations have been documented (Beiras and His, 1994). Sediment geochemical properties determine the type of metal bindings and its trend to desorb, while factors such as pH and salinity can also determine the bioavailability of chemicals bound to sediments (Riba et al., 2004b). Also organic matter affects metal speciation (Lorenzo et al.,

2002) and plays a major role binding different contaminants and may be the responsible of the negative correlation coefficients between organic matter content, concentrations of organic contaminants and elutriate toxicity.

To elucidate the relative performance of elutriate tests on a whole battery of bioassays a comparative study on solid-phase and elutriate toxicity was performed on the results through the percentage of agreement between methodologies (Fig. 2). The toxicity endpoint to sea urchin embryos was in agreement with toxicity to amphipods for 36% of samples (n=22) and this percentage increases to 46 when compared with the results of the test using polychaetes (n=16). The test using amphipods was more sensitive than the test using sea urchin embryos (77.2% to 31.8% respectively) but, on the contrary, embryotoxicity seems a more sensitive endpoint than the bioassay using polychaetes (42% and 25% respectively). The percentages of agreement reported in this study are lower than the percentages of agreement reported by Bay et al. (2003) for pore water toxicity tests (54%) even if the use of this aqueous extract can report higher toxicities than sediment elutriates according to previous results on urban harbour sediments and uncontaminated reference locations (McDonald, 2005). When addressing sediments falling into category II, which are needed of further biological assessment to decide whether they are suitable for open water disposal or not, elutriate tests reported a considerably lower incidence of toxicity than the amphipod bioassay (14.3 and 88.5% respectively) and only a 42.9% of agreement (sediments considered similarly independently of the measurement endpoint considered). This means that the selection of one or another exposure route can influence critically the classification of dredged sediments as toxic or not toxic. Bay et al. (2003) pointed out that pore water toxicity tests are much more likely than solid phase tests to detect toxicity due to 1) the different sensitivity to contaminants among species, 2) variations in contaminant exposure or bioavailability related to the test method, and 3) the influence of naturally occurring toxicants. According to our results solid phase bioassays are more likely than elutriate tests to detect toxicity and evidence the importance of the variations in contaminant exposure and bioavailability related to this extraction procedure. The solid phase bioassays were better correlated with the sediment contamination and this may indicate that empirically-derived SQGs are better predictors of toxic effects in some benthic organisms (Long et al., 2000). Nonetheless the suitability of such guidelines for predicting elutriate toxicity seems compromised thus elutriate tests are further justified in the context of navigational dredging to mimic sediment resuspension scenarios and to study direct water column effects.

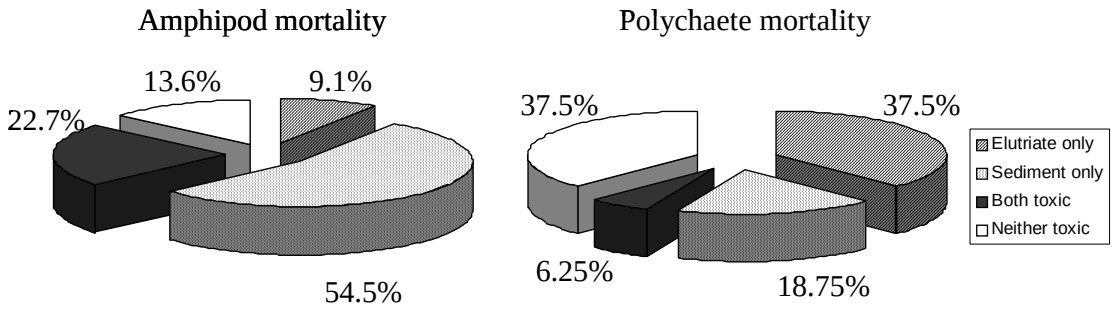

Figure 2

Comparative response of elutriate embryogenesis success and solid-phase toxicity tests of the sediment samples.

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Capítulo 4.

Comparación interlaboratorio de bioensayos de toxicidad

De acuerdo con el paradigma que constituye el desarrollo de ensayos de toxicidad para ser utilizados en un contexto regulador, descrito en el capítulo anterior, se establece la necesidad de realizar ejercicios de intercalibración con diferentes laboratorios que permita evaluar la viabilidad de los bioensayos probados dentro de un protocolo único de caracterización de la toxicidad de materiales de dragado. Los estudios interlaboratorio se utilizan normalmente para evaluar la variabilidad estadística (precisión) entre laboratorios, y sirven también para determinar los factores que contribuyen de forma más determinante en esta variación. En general se considera que los factores de variabilidad más importantes para los bioensayos de toxicidad son (Dillon, 1994):

1. La experiencia y la habilidad del operador,
2. El tipo de instrumentación y calibración y
3. El ambiente en el que se desarrolla el ensayo.

Además de estos factores hay que considerar otros factores abióticos introducidos por la utilización de distinta agua de mar, por ejemplo, y en especial los factores biológicos, introducidos por la utilización de organismos procedentes de distintas poblaciones y que por lo tanto pueden ofrecer una respuesta distinta por una distinta manipulación o estado fisiológico de los organismos.

Los estudios interlaboratorio se han utilizado previamente para validar el uso de diferentes ensayos de toxicidad en distintos países como Estados Unidos, Canadá y Holanda. En estos estudios interlaboratorio se han minimizado, en mayor o menor medida, estos factores mediante el suministro de los individuos de una misma población, con lo que disminuye la influencia de los factores biológicos, utilizando el mismo agua de mar, además de realizarse sobre los mismos materiales de dragado y con un protocolo único y exhaustivo del ensayo de toxicidad (i.e. Stronkhorst, 2003).

Como se describe en el trabajo de introducción (trabajo VII), los ejercicios interlaboratorio que se presentan en este capítulo están también diseñados para evaluar la variabilidad de los resultados obtenidos en distintos laboratorios, aunque se ha permitido un mayor grado de libertad a cada uno de los laboratorios que en estudios precedentes. Se ha considerado más realista que los laboratorios sigan sus propias metodologías a la hora de evaluar la calidad ambiental de los materiales de dragado. No obstante, se han estudiado los factores que necesitarían una mayor homogeneización en los protocolos de ensayo antes de ser considerados para su utilización en un contexto regulador. Este ejercicio se realizó utilizando el ensayo de screening Microtox® sobre la fase sólida del sedimento, el ensayo de desarrollo larvario con embriones del erizo *Paracentrotus lividus*, el ensayo con juveniles de almeja, y el ensayo con anfípodos de distintas especies previamente utilizadas para evaluar la calidad de sedimentos en los laboratorios participantes (trabajos VIII a XI). El número de participantes varió para cada bioensayo dependiendo del los laboratorios que disponían de la tecnología adecuada y la voluntad de contrastar ésta. La Universidad de Cádiz se encargó del diseño del estudio y de la distribución de las muestras, que fueron enviadas a los laboratorios participantes con un protocolo que incluía las guías básicas a seguir. Una vez realizados los ensayos en cada uno de los laboratorios, los resultados fueron enviados a la Universidad de Cádiz para ser estudiados de forma conjunta, comparar los resultados obtenidos con la metodología utilizada y estudiar así los factores determinantes en la variabilidad de cada ensayo.

Tras una primera fase de ensayo, se identificaron un mayor número de factores de variabilidad para los ensayos Microtox® y de desarrollo larvario con embriones del erizo de mar. Tras ser correctamente identificados estos factores y homogeneizados los protocolos de los distintos laboratorios, se volvió a realizar una nueva fase de ejercicios de intercalibración para estos dos ensayos. En cuanto al ensayo de inhibición de la luminiscencia, se consideró el estudio por tres de los laboratorios de un nuevo protocolo para la fase sólida, el denominado 'Basic Solid Phase Test' (BSPT). Este nuevo protocolo

desarrollado por la casa comercial que suministra el aparato ha obtenido resultados similares en el análisis de sedimentos y parece más sencillo y más económico. Para el ensayo de desarrollo larvario con embriones del erizo de mar, se consideró el proceso de obtención de las fases líquidas sobre las que se realiza el ensayo un posible factor importante de variabilidad. Así, en esta segunda fase se realizó el proceso de lixiviación en el laboratorio de la Universidad de Cádiz y se envió con las seis muestras de sedimento una muestra de lixiviado suficiente para realizar el ensayo de forma paralela y comparar los resultados con los obtenidos para los lixiviados realizados por cada laboratorio en sus instalaciones.

Los resultados para el ensayo Microtox® SP, resumidos en el trabajo VIII, ofrecieron una variabilidad entre laboratorios similar a otros ejercicios realizados en otros países previamente a la introducción de este ensayo en las recomendaciones para la gestión de dragados portuarios. El mayor factor de variabilidad entre los distintos laboratorios fue el tiempo de realización del ensayo. Los valores anómalos respecto al resto de laboratorios correspondían a los laboratorios que realizaron el ensayo después de un mes de almacenamiento de los sedimentos. Además este factor parece ser el mayor factor de confusión a la hora de comparar los resultados de distintos laboratorios y también es el causante de la mitad de la variabilidad entre laboratorios (los coeficientes de variación se reducen del 64-114% al 45-75%). Los resultados para el nuevo protocolo sobre fase sólida BSPT mostraron unos resultados entre laboratorios más homogéneos en la clasificación de las muestras y en los valores de toxicidad registrados. Se registró una gran variabilidad para muestras de arenas y en otra muestra que pudo verse afectada por una mala homogeneización de los sedimentos en el proceso de envío. Aunque este protocolo es de nueva aplicación, los resultados obtenidos en esta primera aplicación sobre materiales de dragado son incluso más satisfactorios que los obtenidos para el protocolo antiguo, con unos coeficientes de variación para el resto de muestras de entre el 13 y el 22%. Si se tiene en cuenta que este nuevo diseño es más simple y reduce considerablemente el coste por muestra ensayada respecto al protocolo anterior, parece un ensayo a tener en cuenta a la hora de recomendar un protocolo para su uso en un contexto regulador. La guía de calidad biológica propuesta para este ensayo, establecida en 1000 mg/L expresado en peso seco, parece adecuada para los dos protocolos propuestos aunque parece también recomendable el uso de un sedimento de referencia para las muestras con una proporción de finos (<0.063 mm) menor al 20% como recomienda la agencia canadiense ya que se obtienen las mayores variabilidades entre análisis.

El ejercicio interlaboratorio para el ensayo con embriones del erizo de mar *Paracentrotus lividus* presentado en el trabajo IX mostró unos resultados más variables en la primera fase de estudio, con unos coeficientes de variación entre 25 y 116%. En esta primera fase las principales fuentes de variabilidad se centraban en diferencias fundamentales a la hora de obtener el lixiviado sobre el que se realiza el ensayo, aunque se registró una baja calidad del material biológico en algunos laboratorios (porcentajes de desarrollo normal en el control de toxicidad negativo superiores al 90%). Tras la homogeneización de los protocolos de obtención de los lixiviados la variabilidad entre laboratorios se redujo y ofreció unos resultados considerados satisfactorios en relación a estudios interlaboratorio para este ensayo y otros ensayos biológicos, con unos coeficientes de variación entre 5 y 163%. El mayor factor de variabilidad identificado para este ensayo una vez homogeneizados los protocolos de obtención y realización del ensayo parece ser el estudio de las larvas y especialmente el criterio utilizado para considerar una larva cono normal o anormalmente desarrollada ya que se registran tendencias claras en determinados laboratorios. La guía de calidad biológica propuesta para la clasificación de los materiales de dragado, establecida en 25% de larvas anormales, parece un buen indicador de toxicidad ya que los resultados de todos los laboratorios con porcentajes de larvas anormales superiores eran significativamente distintos a los controles. En cualquier caso parece recomendable el uso de análisis estadísticos tras la corrección de los datos para el valor del control, ya que permiten incluso establecer un doble criterio de toxicidad mediante el uso de distintos valores de p ($p<0.05$ para identificar muestras tóxicas y $p<0.01$ para identificar muestras moderadamente tóxicas).

Como se muestra en el trabajo X, el ensayo con anfípodos obtuvo unos coeficientes de variabilidad entre laboratorios en el rango de los obtenidos en numerosos estudios previos, entre 10 y 81% con un valor medio del 33%. Estos resultados parecen especialmente satisfactorios teniendo en cuenta que se utilizaron especies distintas en la mayoría de los laboratorios participantes y, aunque la metodología fue similar en todos ellos, cada uno de los laboratorios siguió su propia metodología previamente utilizada para la evaluación de la calidad de sedimentos. Como para el ensayo Microtox®, el mayor factor que afectó a la variabilidad entre laboratorios fue el tiempo de almacenamiento de los sedimentos previamente al inicio del bioensayo, que provocó claramente un descenso de la toxicidad registrada, especialmente para muestras afectadas exclusivamente por contaminación de tipo orgánico. La guía de calidad biológica propuesta para considerar una muestra como tóxica o no tóxica podría ser la diferencia de mortalidad respecto al control

del 20-25%, ya que este valor supone para todos los laboratorios la diferencia estadísticamente significativa respecto al control a $p<0.05$. En cualquier caso, parece recomendable el uso de análisis estadísticos para asegurar que esta diferencia es significativa. Si se desea establecer rangos de toxicidad se pueden utilizar distintos criterios estadísticos como se ha descrito anteriormente para el ensayo con embriones de erizo de mar.

En cuanto al ensayo con juveniles de almeja, los resultados presentados en el trabajo XI parecen indicar la dificultad en el uso rutinario de este ensayo para la caracterización de materiales de dragado. El bajo número de laboratorios en los que estaba establecido y la baja sensibilidad al tipo de muestras utilizadas es determinante en los altos coeficientes de variación para la medida de mortalidad a 7 y 14 días, aunque la clasificación de las muestras sea homogénea. En cuanto a la medida subletal de enterramiento, los resultados fueron también muy variables y presentaron dificultades a la hora de su interpretación por la subjetividad a la hora de decidir los individuos que se consideran enterrados y los que no lo están. En este estado de desarrollo no parece adecuado recomendar ninguna guía de calidad para la clasificación de las muestras como tóxicas o no tóxicas, salvo la diferencia significativa a estaciones de referencia y/o control como se utilizan en estudios de calidad ambiental.

El estudio de todos los resultados mediante el análisis multivariante, presentado en el trabajo XI cerrando este capítulo, relacionó las mortalidades registradas por las distintas especies de anfípodos con la mayoría de contaminantes medidos, aunque en especial parece responder a la contaminación de tipo metálica. El otro ensayo sobre la fase sólida, el ensayo con juveniles de almeja, está directamente relacionado con la contaminación por As y Cu aunque estos resultados parecen determinados por la alta toxicidad registrada sólo para los materiales de dragado recogidos en la ría de Huelva. Por el contrario, la respuesta registrada por el ensayo Microtox® SPT apareció relacionada con la contaminación metálica pero también con la proporción de finos y el contenido en materia orgánica de la muestra y especialmente con los contaminantes orgánicos PCBs y PAHs, que aparecen siempre relacionados con estas dos propiedades del sedimento. El protocolo BSPT también se relacionó con el contenido en materia orgánica pero parece menos afectado por la proporción de finos de la muestra que el protocolo anterior. Esto puede ser un indicio de la menor influencia en los resultados de este factor de confusión tan determinante en el otro protocolo estudiado, el SPT. En cualquier caso, este protocolo está también relacionado con la contaminación metálica y especialmente con los contaminantes de tipo orgánico

PCBs y PAHs. Por último, el ensayo de desarrollo larvario se muestra igualmente relacionado con la proporción de finos y el contenido en materia orgánica del sedimento así como con la contaminación de tipo metálica, pero este ensayo parece no relacionarse con la contaminación de tipo orgánica o, más especialmente, relacionado inversamente. Esto pone de evidencia la ausencia de efectos tóxicos en el caso de muestras con un elevado contenido en orgánicos, tanto materia orgánica como contaminación orgánica, establecida por este tipo de ensayos.

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Nota de Investigación/Research Note

Ejercicio interlaboratorio de bioensayos marinos para la evaluación de la calidad ambiental de sedimentos costeros. I. Descripción del ejercicio y calidad de los sedimentos

Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. I. Exercise description and sediment quality

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Resumen

El presente trabajo resume la organización del ejercicio interlaboratorio para distintos ensayos de toxicidad realizados sobre sedimentos de puertos españoles así como los resultados de la caracterización fisicoquímica de las muestras utilizadas. El ejercicio, que incluyó cuatro bioensayos (un ensayo comercial para la evaluación inicial de la toxicidad de los sedimentos, un ensayo para evaluar la toxicidad de los lixiviados y dos ensayos sobre la fase sólida) se desarrolló en dos fases. En la Fase I se distribuyeron a cada uno de los distintos laboratorios participantes seis muestras de sedimentos dragados en diferentes puertos alrededor de la costa española (Barcelona, Bilbao, Cádiz, Cartagena y Huelva) cuya toxicidad fue evaluada mediante los ensayos seleccionados. La Fase II, que incluyó seis nuevas muestras de sedimento (provenientes de los puertos de Algeciras, Bilbao, Cádiz y Huelva), incluyó sólo dos ensayos para los que se habían identificado distintos factores de confusión durante la primera fase del ejercicio. Los resultados de la caracterización química de las muestras arrojaron, en la mayoría de los sedimentos, altas concentraciones de determinados elementos metálicos, principalmente Cu, Hg y en menor medida As y Cd, y de los compuestos bifenilos policlorados medidos. De acuerdo con las concentraciones encontradas y las guías de calidad de sedimentos usadas en España para la gestión de dragados portuarios, cinco de las seis muestras de sedimentos que se usaron en cada fase del ejercicio se considerarían materiales con concentraciones altas de contaminantes y, por ende, su vertido al mar no sería permitido.

Palabras clave: material de dragado, variabilidad interlaboratorio, contaminación.

Abstract

The results of an interlaboratory assessment made to evaluate the use of different bioassays for sediment quality characterization for a regulatory purpose are presented. The objective of the study was to determine the main differences among sediment bioassays conducted at different laboratories and to evaluate how this can influence dredged material management. The exercise consisted of two parts. During Phase I, six dredged sediment samples from different Spanish ports (Barcelona, Bilbao, Cádiz, Cartagena and Huelva) were distributed to different laboratories where they were applied a screening test for initial toxicity evaluation, a test for elutriates and two solid-phase bioassays. In Phase II, six new sediment samples (from the ports of Algeciras, Bilbao, Cádiz and Huelva) were tested to re-evaluate some bioassays for which different interfering factors were identified during the first phase. The chemical characterization results for Phase I showed high concentrations of some compounds in all the samples except one, which could be considered uncontaminated, while those for Phase II showed high concentrations in most sediments of some metallic compounds, mainly Cu, Hg and As, and to a lesser extent Cd, and of the total polychlorinated biphenyl congeners analyzed. According to Spanish guidelines for dredged material management, only one of the six samples assessed in each phase of the exercise would be suitable for open water disposal.

Key words: sediment, round robin, ring test, toxicity, contamination.

Introducción

La Comisión Europea, siguiendo el ejemplo de otras iniciativas de agencias internacionales para el control de los procesos de dragado, incluyó los materiales resultantes de éstos en la Decisión 2000/532/EC, modificada por la Decisión 2001/

Introduction

In 2000, in accordance with international policies and regulations aimed at controlling the disposal of dredged material, the European Commission included in its Decision 2000/532/EC (modified by Decision 2001/118CE) sediments

118CE, como residuos que pueden ser considerados peligrosos si presentan altas concentraciones de contaminantes químicos. En España, aunque la legislación no incluye aún una directiva para regular la caracterización de los procesos de dragado, se han venido utilizando un conjunto de recomendaciones publicadas en 1994 por distintos organismos y entes relacionados (CEDEX 1994). De forma muy resumida estas recomendaciones incluyen la caracterización de los sedimentos a dragar en los casos en los que no se disponga de información suficiente para autorizar o descartar el vertido libre. Esta caracterización incluye la determinación de algunos parámetros normales del sedimento como el tamaño de grano, el contenido en materia orgánica o la determinación de la contaminación microbiológica, así como la cuantificación de determinados contaminantes de interés en la fracción menor a 63 μm. Los resultados de estos análisis se comparan con las correspondientes guías máximas de calidad de sedimentos, denominadas niveles de acción 1 y 2 (NA 1 y NA2; tabla 1), y los sedimentos son así clasificados en las tres categorías de gestión existentes: categoría I para los materiales con concentraciones inferiores al NA 1, categoría II para los materiales que presentan concentraciones entre NA 1 y el NA 2, y categoría III para los sedimentos con concentraciones superiores a NA 2. De este modo, los materiales de categoría I podrían ser vertidos al mar siguiendo un proceso de autorización normal de vertido, los materiales de categoría III necesitarían de un proceso especial de autorización para su gestión y no podrían ser vertidos al mar, y los materiales de categoría II necesitarían de un proceso de caracterización más exhaustivo para poder descartar posibles efectos adversos en el medio acuático y en consecuencia poder autorizar su vertido al mar.

Varios marcos de evaluación nacionales e internacionales para la gestión de materiales de dragado requieren la tradicional caracterización química y, además, la realización de ensayos de toxicidad para la evaluación de posibles efectos biológicos adversos (Environment Australia 2002, RIKZ 2001). En cualquier caso, y como para cualquier otra técnica analítica, si estas técnicas se quieren incorporar en programas reguladores no sólo se necesitan metodologías estandarizadas, sino que el proceso de validación necesario debe incluir además de esta estandarización el estudio de otras características como la precisión intralaboratorio o la variabilidad interlaboratorio. Esta última se considera una medida de la reproducibilidad del método cuando se realiza en distintos laboratorios siguiendo la misma metodología, con el mismo organismo y sobre las mismas muestras (USEPA 1994). Los principales factores que contribuyen a la variabilidad del método y que deberían tenerse en cuenta para la interpretación práctica de los resultados son: (1) los relacionados con el organismo de ensayo (edad, condición, mantenimiento o sensibilidad), (2) los relacionados con el desarrollo del ensayo (diferencias en el material o en la calidad del agua sobrenadante) y (3) los relacionados con el personal que desarrolla los ensayos, como por ejemplo su experiencia.

from dredging activities among the residues hazardous for disposal at sea if their chemical content of specific compounds is high. In Spain, even though there is no specific law for the characterization of dredged materials, both public and private agencies have enacted specific recommendations (CEDEX 1994) that have been routinely used for dredged material management. Based on these recommendations, dredged material is first analyzed to measure some conventional sediment parameters (e.g., grain size distribution, organic matter content, microbiological contamination) and to quantify some contaminants of concern in the <63-μm fraction. The results of these analyses are then compared against a list of reference values, corresponding to the low and high sediment quality guidelines, named Action Levels 1 and 2 (table 1). Finally, sediments are classified into three different categories: category I for materials with all concentrations lower than Action Level 1 (AL1), category III for materials with all concentrations higher than Action Level 2 (AL2), and category II for materials with concentrations between AL1 and AL2. Category I materials are considered suitable for open water disposal and follow a normal authorization process, category III materials need a special authorization process and are not considered suitable for open water disposal, and category II materials need to be further analyzed for classification into either category I or III.

Several national and international assessment frameworks for dredged material management require laboratory toxicity tests in addition to the traditional chemical characterization (Environment Australia 2002, RIKZ 2001). A standardization of methods is required as part of the validation of any analytical technique if biological tests have to be incorporated into regulatory programs (acceptable interlaboratory variability, intralaboratory precision). Interlaboratory precision (also referred to as round robin or ring tests) indicates

Tabla 1. Niveles de acción (NA) usados para caracterizar material de dragado en España (CEDEX 1994). Las concentraciones están medidas en la fracción de sedimento <63 μm.

Table 1. Action Levels used in Spain for dredged material management (CEDEX 1994). Concentrations in the <63-μm sediment fraction.

Components	Action Level 1 (mg kg^{-1} dry weight)	Action Level 2 (mg kg^{-1} dry weight)
Hg	0.6	3.0
Cd	1.0	5.0
Pb	120	600
Cu	100	400
As	80	200
Cr	200	1000
Ni	100	400
Zn	500	3000
Σ_7-PCBs	0.03	0.1

Para este ejercicio interlaboratorio se incluyeron cuatro bioensayos seleccionados de acuerdo con la experiencia de los laboratorios y con resultados previos en la caracterización de materiales de dragado y/o sedimentos contaminados (DelValls *et al.* 2003). Debido a su estandarización y amplio uso en la evaluación de muestras ambientales, se seleccionó el aparato comercial Microtox® como herramienta para la evaluación inicial de la toxicidad, siguiendo el protocolo para el ensayo sobre fase sólida (SPT). Se incluyeron también el ensayo de supervivencia con crustáceos anfípodos, el ensayo sobre la fase sólida de enterramiento y mortalidad con juveniles de almeja y el ensayo con estadios larvarios de erizo de mar de 48 h de duración sobre el lixiviado de los sedimentos. Los objetivos principales para el desarrollo del ejercicio fueron:

- Determinar las diferencias en las respuestas registradas por los distintos laboratorios, es decir, evaluar la precisión de cada uno de los bioensayos para caracterizar la toxicidad de materiales de dragado cuando los ensayos se realizan por un operador diferente y en laboratorios diferentes.

- Identificar los factores de confusión principales que afectan el desarrollo y la interpretación de los resultados cuando éstos se realizan en laboratorios con y sin experiencia previa en este tipo de bioensayos.

- Discutir las posibles causas de la variabilidad interlaboratorio con la intención de mejorar la estandarización de protocolos y el uso de los bioensayos como herramienta complementaria a las guías químicas para la caracterización de materiales de dragado y su gestión.

- Discutir el uso de las guías biológicas propuestas previamente para considerar las muestras como tóxicas o no tóxicas según cada ensayo.

Organización del ejercicio interlaboratorio

Todos los laboratorios participaron voluntariamente y de acuerdo a su experiencia previa en la aplicación de los ensayos, por lo tanto el número de participantes fue diferente en cada caso. Algunos de los participantes han usado este tipo de ensayos para el análisis rutinario de la toxicidad de sedimentos desde hace varios años pero otros sólo los han incorporado recientemente. Los resultados de cada laboratorio fueron identificados con un número para respetar el anonimato.

Los puertos y las estaciones de muestreo fueron seleccionadas por el CEDEX y la Universidad de Cádiz (DelValls *et al.* 2003). La Universidad de Cádiz distribuyó una alícuota de cada muestra (identificada de la A a F) a los laboratorios participantes y un protocolo donde se describían los parámetros y las condiciones necesarias para el desarrollo de los ensayos de toxicidad. Los cambios introducidos por cada laboratorio al protocolo original recibido se enviaron con los resultados.

Debido a distintos factores de confusión encontrados durante el primer ejercicio (descritos en Casado-Martínez *et al.* how reproducible a method is when conducted by a large number of laboratories using the same methodology on the same organism and samples (USEPA 1994). The following interfering factors have already been identified and should be taken into account for the practical interpretation of the results: (1) factors related to the test organism (age, condition, handling or sensitivity); (2) factors related to the test set-up (differences in the material or in the quality of the overlying water); and (3) factors related to the technicians conducting the tests.

This interlaboratory exercise included four bioassays selected according to the experience of the laboratories and to previous results for dredged material and/or contaminated sediments (DelValls *et al.* 2003). The commercial solid-phase Microtox® test was chosen as screening tool due to its standardization and wide use in environmental toxicity assessment. The exercise also included a whole sediment survival bioassay using amphipods, a whole sediment burrowing and survival bioassay using juvenile bivalves and a 48-h sea urchin embryo-larval development bioassay performed on sediment elutriates. The main objectives were:

- To determine the differences in mean responses among laboratories, that is, to assess the precision of each of the bioassays to characterize dredged material toxicity when developed by different operators and laboratories.

- To identify the main interfering factors affecting test results and their interpretation when they are developed by different laboratories with and without demonstrated previous experience in the same bioassays.

- To discuss the possible causes of interlaboratory variation to improve the standardization of the protocol and the use of bioassays as a complementary tool to chemical guidelines for dredged material characterization and management.

- To discuss the use of previously derived or proposed biological guidelines to consider a sample toxic or not toxic.

Study design

The number of participants differed in each of the bioassays because not all the laboratories were properly equipped to perform them all. Laboratories participated voluntarily and had some previous experience in applying these tests; however, while some laboratories had used some tests in routine analysis for years, others had only recently included toxicity bioassays in their routine. Each laboratory was identified with a number to maintain anonymity.

In Phase I of the excercise, CEDEX and Cádiz University selected six sampling stations (DelValls *et al.* 2003). One sediment sample from each station (named A to F) was then sent to the laboratories together with a protocol describing the test

2006a, b) se realizó una segunda fase en marzo de 2004, en la que sólo se incluyeron los ensayos con Microtox® y con estadios larvarios de erizo de mar. La organización de esta fase (II) fue similar a la previa (Fase I) pero las muestras se recogieron en distintas estaciones. Con las muestras se envió un protocolo más completo con especial énfasis en los factores de confusión encontrados durante la Fase I y que se discutieron, previamente a la Fase II, con los laboratorios participantes.

Sedimentos estudiados

Las muestras de sedimento distribuidas en la Fase I del ejercicio (abril de 2003) se recogieron en cinco puertos localizados alrededor de la costa española (fig. 1). Las muestras incluían un sedimento control (muestra A) proveniente de una zona intermareal de la Bahía de Cádiz que, de acuerdo con estudios previos (DelValls *et al.* 1997), está libre de contaminación y no presenta toxicidad. Los sedimentos identificados como muestra B también provenían de la Bahía de Cádiz pero de la zona interior del puerto, posiblemente con contaminación de tipo orgánico. La muestra C se recogió en el puerto de Huelva donde se han encontrado previamente niveles altos de distintos compuestos metálicos (Riba *et al.* 2004). Las muestras D y E provenían de los puertos de Bilbao y Barcelona, respectivamente, cuya fuente de contaminación principal es el transporte marítimo. La muestra F eran sedimentos provenientes del puerto de Cartagena, afectado por actividades mineras de importancia histórica.

Para la Fase II del ejercicio de intercalibración, las muestras A' y B' se recogieron aproximadamente en el mismo lugar que el año anterior. Las muestras C' y D' fueron recogidas en el puerto de Huelva y la F' en el puerto de Bilbao (fig. 1). Los sedimentos de la muestra E' provenían del estuario del Río Guadarranque, en la zona de la Bahía de Algeciras y afectado principalmente por contaminación de tipo orgánico.

Las muestras de los sedimentos se recogieron con una draga tipo Van Veen y se agruparon en contenedores de polipropileno refrigerados (50 L). Las muestras de sedimento fueron homogeneizadas y tamizadas a través de una malla de 0.5 mm para retirar organismos y restos de ellos que pudieran interferir en los ensayos, dividiéndolas en alícuotas de distinto tamaño según el número y tipo de ensayos que fuese a realizar el laboratorio de destino. Antes de los ensayos, las muestras se conservaron herméticamente cerradas en oscuridad y a 4ºC. Estas condiciones de conservación se alteraron durante el envío de las muestras, que se realizó en neveras refrigeradas y duró alrededor de 24 h.

Caracterización de los sedimentos

La caracterización de los sedimentos usados para los ensayos de toxicidad siguió las recomendaciones españolas usadas actualmente para materiales de dragado (CEDEX 1994). Esta caracterización incluyó algunos parámetros normales del sedimento (distribución de tamaños de grano o

conditions. The laboratories reported any change to the initial protocol. Since different interfering factors were identified during Phase I (Casado-Martínez *et al.* 2006a, b), a second phase (Phase II) was carried out in March 2004 to refine the results. In Phase II only the Microtox® test and the bioassay using sea urchin embryo-larval stages were included in the exercise. The study design was similar to that of Phase I but some of the test sediments were obtained from different sites. An updated protocol, reporting the interfering factors found during the previous study, was sent together with the samples to the laboratories.

Test sediments

For Phase I, one control sediment and five contaminated dredged sediment samples were collected from different Spanish ports in April 2003 (fig. 1). The control sediment (sample A) was collected from an intertidal zone of Cádiz Bay, considered an uncontaminated area according to previous studies (DelValls *et al.* 1997). Sample B was also collected at Cádiz Bay but from the inner part of the port, probably contaminated by organic compounds. Sample C was collected from the port of Huelva, where high levels of metallic contamination due to mining activities and industrial sewage have been found (Riba *et al.* 2004). Samples D and E were collected from the ports of Bilbao and Barcelona, respectively, because of the high maritime transport and shipping activities that characterize both. Sample F was collected from the port of Cartagena, where metal concentrations are very high as a result of historically important mining activities.

For Phase II of the intercalibration exercise, samples A' and B' were collected from the same stations in Cádiz Bay as the previous year. Samples C' and D' were collected from the port of Huelva. Sample E' was collected from the Guadarranque River estuary, located in Algeciras Bay, because of the hydrocarbon contamination of the area. Sample F' was collected from the port of Bilbao (fig. 1).

Sediments were collected with a Van Veen grab and pooled in polypropylene containers (50 L). Prior to the analyses, the sediment samples were kept hermetically closed in darkness and at 4ºC. Samples were homogenized and sieved through a 0.5-mm mesh to remove organisms and other debris that could interfere in the tests, and then divided into aliquots of different size depending on the number and type of tests to be carried out in each laboratory. All samples were distributed to the laboratories in iceboxes in less than 24 h.

Chemical analyses

The chemical characterization of the sediments used for the toxicity bioassays followed the Spanish recommendations for dredged material (CEDEX 1994). This characterization included the measurement of some conventional parameters of the sediment (grain size distribution and organic matter content), as well as the quantification of As, Cd, Cu, Cr, Hg, Ni, Pb

contenido en materia orgánica) y la cuantificación de As, Cd, Cu, Cr, Hg, Ni, Pb y Zn, y algunos compuestos de tipo orgánico como la suma de determinados bifenilos policlorados (PCBs) e hidrocarburos policíclicos aromáticos (PAHs) en la fracción menor a 63 µm, previamente secada a 40ºC. La distribución de los tamaños de grano se realizó según la UNE 103 101, y el contenido en materia orgánica se estimó por el método de pérdida por ignición (LOI) a 550ºC y posterior determinación gravimétrica, como se recomienda para dragados de pequeños volúmenes (CEDEX 1994).

Las especies metálicas se determinaron previa digestión ácida en horno microondas en frascos de Teflón. Para la determinación del Hg se utilizó la técnica de generación de vapor frío y para la del As la generación de hidruros, ambos cuantificados mediante espectrometría de absorción atómica. Las concentraciones de Cd, Pb, Cu, Zn y Cr se determinaron mediante espectrometría de absorción atómica con llama o en horno de grafito dependiendo de las concentraciones en los sedimentos. Los PCBs e PAHs se cuantificaron, previa extracción con ciclohexano y diclorometano, mediante tratamiento con ultrasonido y concentración y limpieza por columna cromatográfica. La determinación de PCBs (suma de 7 compuestos) se realizó por cromatografía de gases acoplada a un detector por captura de electrones (de acuerdo con USEPA, método 8080) y los PAHs (suma de 16 compuestos) se determinaron mediante cromatografía líquida de alta resolución con detección por fluorescencia (de acuerdo con la USEPA, método 8310). Todos los análisis se comprobaron con materiales de referencia (Material Certificado de Referencia NRC-CNCR 277 BCR para metales y NRC-CNRC HS-1 para compuestos orgánicos) y estaban dentro del rango de aceptabilidad.

Resultados

Los resultados de los análisis químicos y otros parámetros del sedimento se incluyen en las tablas 2 y 3. Las concentraciones químicas encontradas en los sedimentos se compararon con los valores límites (NA 1 y NA 2) usados para la caracterización de materiales de dragado en España (CEDEX 1994) y se clasificaron de acuerdo a las distintas opciones de gestión (tabla 4). La muestra A, que podría ser considerada un control negativo de toxicidad (DelValls *et al.* 1997), era un sedimento arenoso con bajo contenido en materia orgánica. Como se esperaba, las concentraciones de compuestos químicos fueron bajas y en ningún caso superan los niveles de acción. El resto de las muestras presentaron un alto porcentaje de finos de 57–60% (muestras B, E y F) a 90% (estación C). Los sedimentos también tuvieron un alto contenido en materia orgánica que varió entre 10% (muestras C y F) y 17% (muestra E). De acuerdo con estos resultados todos los sedimentos excepto los de la estación A no serían aptos para su vertido al mar. Las muestras B y D excedieron el NA 2 para los PCBs. La muestra C estuvo principalmente afectada por contaminación de tipo metálica y superó los NA 2 correspondientes a las especies

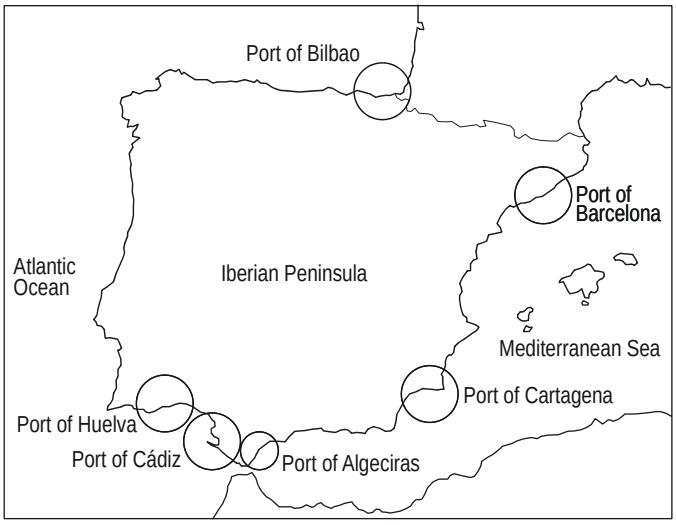

Figura 1. Localización de los puertos españoles seleccionados para el ejercicio interlaboratorio.

Figure 1. Location of the Spanish ports selected for the interlaboratory exercise.

and Zn, and of some organic compounds such as the sum of certain congeners of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in the <63-µm fraction. For the analyses, sediments were dried at 40ºC for 24 h. Grain size distribution followed UNE 103 101 and organic matter content was estimated by loss of ignition (LOI) at 550ºC and gravimetric determination as recommended for small dredged volumes (CEDEX 1994).

Metals were determined in microwave acid-digested samples in Teflon vessels. The cold vapor technique was used for Hg and hydride generation for As, both quantified using atomic absorption spectrometry. The concentrations of Cd, Pb, Cu, Zn and Cr were determined using flame or furnace atomic absorption spectrometry, depending on the metal content. The PCBs and PAHs were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment and concentration and clean-up with column chromatography. Determination of PCBs (sum of 7 congeners) was made with gas chromatography with electron capture detection (GC-ECD) (following USEPA, method 8080), while PAHs (sum of 16 congeners) were determined using HPLC with fluorescence detection (following USEPA, method 8310). All the analytical procedures were tested with reference materials (Certified Reference Material, NRC-CNRC 277 BCR for metals; and NRC-CNRC HS-1 for organic compounds) and were within the range of acceptability.

Results

The results of the chemical analyses and other sediment parameters are shown in tables 2 and 3. The sediment chemical concentrations were compared to the limit values (named Action Levels, table 1) used in Spain for dredged material characterization (CEDEX 1994), and the samples were

Tabla 2. Resultado de los análisis químicos y parámetros normales del sedimento de las diferentes muestras (A–F) usadas en la Fase I de este estudio. Todas las concentraciones están expresadas en mg kg^{-1} de peso seco, excepto la suma de PCBs, expresada en μg kg^{-1} peso seco, n.d. = no detectado.
Table 2. Results of the chemical analyses and normal parameters of the sediment samples (A–F) used in Phase I of this study. All concentrations are given in mg kg^{-1} dry weight, except for the sum of the PCB congeners, given in μg kg^{-1} dry weight; n.d. = not detected.

	A	B	C	D	E	F
% coarse	0.19	0.05	0.19	2.39	1.41	3.95
% sand	99.8	40.4	9.60	20.3	39.9	38.2
% fine sediment	0.04	59.5	90.2	77.3	58.7	57.8
% OM	1.07	13.8	10.6	14.8	17.6	10.6
% dry weight	84.1	56.8	41.8	53.7	33.4	55.7
As	3.42	30.8	531	67.3	29.0	101
Cd	0.92	1.32	2.50	2.00	2.88	98.5
Cr	0.10	14.9	24.1	18.3	93.9	66.6
Cu	6.98	203	1497	102	601	665
Hg	0.05	1.98	1.99	0.74	4.12	136
Ni	0.06	20.1	7.10	26.4	32.3	29.0
Pb	2.28	86.9	384	147	455	1397
Zn	21.3	378	1857	476	1165	8651
Σ_7-PCBs	n.d.	144	n.d.	111	273	123
Σ_{16}-PAHs	n.d.	n.d.	n.d.	66.7	1.80	0.91

metálicas As y Cu, y en las otras dos muestras, E y F, se registró una mezcla de compuestos de tipo orgánico (PCBs) e inorgánico (Hg y Cu y también Cd para la muestra E) que excedían los correspondientes NA 2. Sólo los sedimentos de la estación D presentaban una concentración importante de PAHs.

Del análisis de los sedimentos distribuidos en la Fase II del ejercicio se obtuvieron resultados similares: con excepción de la muestra A', el resto de los sedimentos fueron clasificados en la Categoría III ya que la concentración de determinados compuestos excedió los correspondientes NA 2. Las muestras del puerto de Huelva (C' y D') estuvieron principalmente afectadas por As, Cd y Cu. Las muestras E' y F' se clasificaron como no aptas para su vertido al mar debido a sus altas concentraciones de PCBs, aunque también presentaron importantes concentraciones de PAHs y de As y Hg, que superaban los NA 1. El contenido en material orgánico de los sedimentos también fue elevado, entre 6% y 15%. Las muestras C' y D' mostraron una carga química similar aunque esta última tenía un porcentaje de finos mayor y el doble contenido en materia orgánica que la primera.

Discusión

De acuerdo con las características fisicoquímicas determinadas en los sedimentos analizados, son de esperarse niveles bajos de toxicidad para la muestra A, lo que permitiría vertirla al mar sin mayor problema tras un proceso normal de autorización; sin embargo, las otras cinco muestras serían clasificadas

classified according to the required management options (table 4). Sample A, considered a negative control of toxicity (DelValls *et al.* 1997), was a sandy sediment with very low organic matter content. As expected, all chemical concentrations were low and far from the limit values, except for Cd that was closer. The rest of the samples showed a high percentage of fine sediments, ranging from 57–60% in samples B, E and F to 90% in sample C. Organic matter content ranged from 10% in samples C and F to 17% in sample E. According to the chemical analyses, all the samples except for sample A would be considered not suitable for open water disposal. The concentrations of PCBs in samples B and D were responsible for their category III classification. Sample C was only affected by metallic contamination, while As and Cu failed AL2. For samples E and F, a mixture of organic (PCBs) and inorganic species (Hg and Cu, and for E also Cd) exceeded the corresponding AL2. The concentrations of PAHs were low in samples E and F and high only in sample D; they were not detected in samples A, B and C.

All the samples used in the Phase II of the study except for sample A' were classified in category III because some compounds failed AL2. The samples from the port of Huelva were mainly contaminated with As, Cd and Cu. Samples E' and F' were classified as not suitable for aquatic disposal because the concentrations of PCBs were higher than the corresponding AL2; moreover, the concentrations of PAHs were high. Organic matter content was very variable and ranged from 6% to 15%, the latter value corresponding to sample F'. Samples C' and D' showed similar concentrations of chemical

Tabla 3. Resultado de los análisis químicos y parámetros normales del sedimento de las diferentes muestras (A'–F') usadas en la segunda fase de este estudio. Todas las concentraciones expresadas como mg kg^{-1} en base al peso seco, excepto la suma de PCBs, expresada como μg kg^{-1} peso seco; n.d. = no detectado.
Table 3. Results of the chemical analyses and normal parameters of the sediment samples (A'–F') used in Phase II of this study. All concentrations are given in mg kg^{-1} dry weight, except for the sum of the PCB congeners, given μg kg^{-1} dry weight; n.d. = not detected.

	A'	B'	C'	D'	E'	F'
% coarse	0.31	0.04	0.03	0.24	0.05	38.1
% sand	99.0	42.9	56.0	8.36	5.00	14.5
% fine sediment	0.68	57.1	44.0	91.4	94.9	47.4
% OM	1.50	14.0	6.30	9.37	9.00	15.1
% dry weight	80.0	60.8	41.8	55.5	49.8	30.5
As	3.98	33.7	273	336	109	104
Cd	0.87	2.00	1.32	1.03	0.17	2.00
Cr	0.22	16.3	8.13	12.1	38.2	23.1
Cu	8.67	197	772	497	5.01	204
Hg	0.09	1.54	1.20	0.90	1.90	1.43
Ni	0.07	23.9	129	6.18	74.7	32.0
Pb	3.98	97.4	217	210	21.6	285
Zn	24.5	405	1176	974	138	777
Σ_7-PCBs	n.d.	129	n.d.	2.29	109	256
Σ_{16}-PAHs	n.d.	n.d.	n.d.	n.d.	4.21	13.9

Tabla 4. Clasificación de las muestras según las recomendaciones en uso en España (CEDEX 1994) y los requerimientos para cada material.
Table 4. Classification of samples according to the recommendations in use for dredged material management in Spain (CEDEX 1994) and the requirements for each material.

Sample	Category	Management requirements
Phase I		
A	I	Suitable for aquatic disposal; only mechanical effects should be taken into account
B	III	Not suitable for aquatic disposal; PCB values higher than Action Level 2
C	III	Not suitable for aquatic disposal; As and Cu higher than Action Level 2
D	III	Not suitable for aquatic disposal; PCB values higher than Action Level 2; high PAH concentrations
E	III	Not suitable for aquatic disposal; Cu, Hg and PCB values higher than Action Level 2
F	III	Not suitable for aquatic disposal; Cd, Cu, Hg and PCB values higher than Action Level 2
Phase II		
A'	I	Suitable for aquatic disposal; only mechanical effects should be taken into account
B'	III	Not suitable for aquatic disposal; PCB values higher than Action Level 2
C'	III	Not suitable for aquatic disposal; As, Cd, Cu and Ni higher than Action Level 2
D'	III	Not suitable for aquatic disposal; As, Cd and Cu higher than Action Level 2
E'	III	Not suitable for aquatic disposal; PCB values higher than Action Level 2; high PAH concentrations
F'	III	Not suitable for aquatic disposal; PCB values higher than Action Level 2; high PAH concentrations

como categoría III, cuyo vertido en principio se prohibe por ser consideradas potencialmente tóxicas. No obstante estar afectadas por distintos tipos de contaminación, para poder vertirlas al mar todas requerirían de un procedimiento de autorización especial. Además, estas cinco muestras eran sedimentos finos con alto contenido en materia orgánica. Los resultados para la serie de muestras de la Fase II fueron similares y su clasificación en categorías de gestión resultó igual: todas las muestras excepto el sedimento arenoso (A') fueron clasificadas como categoría III, es decir, con efectos adversos potenciales.

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compounds but differences in the sediment characteristics: the organic matter content of D' was double that of C', and D' also had a higher percentage of fine sediments.

Discussion

According to the physicochemical characteristics found in the sediments analized, low or no toxicity is expected from sample A, which is sandy sediment suitable for open water disposal after a normal authorization process. The rest of the samples fell into category III and potential toxicological effects are therefore expected, though differences in their type of contamination were found. This group of five samples consisted of fine sediments with high organic matter content and their chemical concentrations are high enough to be considered potentially toxic, thus requiring a special authorization process for open water disposal. The results obtained from the Phase II samples were similar and their classification according to management categories was the same: all samples except for sample A', the sandy sediment, were classified as category III, with potential biological effects.

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Nota de Investigación/Research Note

Ejercicio interlaboratorio de bioensayos marinos para la evaluación de la calidad ambiental de sedimentos costeros en España. II. Ensayo de inhibición de la bioluminiscencia para la evaluación rápida de la toxicidad de sedimentos

Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. II. Bioluminescence inhibition test for rapid sediment toxicity assessment

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Resumen

El presente trabajo resume el ejercicio interlaboratorio realizado para evaluar la variabilidad del ensayo Microtox® sobre fase sólida. El ejercicio se dividió en dos fases consecutivas, cada una de ellas desarrollada sobre seis muestras de sedimento de puertos españoles. La Fase I incluyó seis laboratorios y se obtuvieron resultados para el protocolo SPT (*Solid Phase Test*). La Fase II incluyó siete laboratorios y, además de obtenerse resultados del protocolo SPT, tres de ellos utilizaron también el protocolo para la fase sólida BSPT (*Basic Solid Phase Test*). Si bien se han identificado factores que pueden tener un cierto efecto sobre los resultados, y por lo tanto en la clasificación de las muestras, los coeficientes de variación obtenidos pueden considerarse entre los valores normales para ensayos biológicos. Si esto se tiene en cuenta para el análisis de los resultados, especialmente para los de la Fase I, la variabilidad baja considerablemente. El protocolo BSPT obtuvo resultados más consistentes que el protocolo SPT aun contando sólo con los resultados de un número reducido de laboratorios. La mayor simplicidad del ensayo pudo ser la causa de esta menor variabilidad.

Palabras clave: Microtox®, material de dragado, *Vibrio fischeri*, variabilidad interlaboratorio.

Abstract

The Microtox® bioassay was tested in an interlaboratory study to evaluate the variability when using solid-phase samples. The exercise consisted of two consecutive phases each one carried out with six sediment samples from Spanish ports. Phase I included six laboratories that reported results for the solid-phase test (SPT) protocol, while Phase II included seven laboratories for the SPT protocol and three laboratories for the basic solid-phase test (BSPT) protocol. Even though some interfering factors were identified that could affect the results and sample classification, the coefficients of variation (CV) can be considered in the range for biological tests. When these factors are considered for further assessments, interlaboratory variability is significantly reduced. The BSPT showed lower CV values than the SPT despite the small number of participating laboratories. This better performance may be explained either by the simplicity of the methodology.

Key words: Microtox®, round robin test, dredged material, *Vibrio fischeri.*

Introducción

El ensayo de bioluminiscencia utiliza un cultivo estandarizado de una bacteria marina, *Vibrio fischeri* NRRL B-11177

Introduction

The bioluminescent bacterial test uses a standardized culture of a selected strain of a marine bacterium, *Vibrio fischeri*

(previamente conocida como *Photobacterium phosphoreum*), cuya emisión de luz puede medirse. La luz es emitida como resultado de un ciclo metabólico unido a la respiración celular y, por lo tanto, una interrupción de la respiración celular normal tiene como consecuencia una disminución inmediata de la emisión de luz. Este ensayo se desarrolló inicialmente para evaluar la toxicidad aguda de muestras de agua y aguas residuales así como para extractos (Bulich 1979). Posteriormente este protocolo inicial fue utilizado para la evaluación de la toxicidad de sedimentos mediante la exposición de las bacterias a los lixiviados, al agua intersticial o a extractos con disolventes orgánicos. Aunque este protocolo de ensayo fue ampliamente utilizado para caracterizar sedimentos (Demuth *et al.* 1993, Carr *et al.* 1996), más recientemente se ha adaptado como ensayo sobre la fase sólida. De esta manera, este nuevo protocolo incorpora el contacto directo de las bacterias con las partículas de sedimento, así como con el agua intersticial (Microbics 1992).

El ensayo sobre la fase sólida, denominado Microtox® Solid Phase Test (SPT) fue desarrollado por AZUR Environmental (Carlsbad, CA, USA) y se ha usado durante los últimos años en distintos estudios para evaluar toxicidad aguda (Stronkhorst 2003, Riba *et al.* 2004). El protocolo SPT mide la emisión de luz de las bacterias después de su incubación en contacto directo con una mezcla de sedimento y disolvente, y tras haber sido recuperadas mediante la filtración de la mezcla en estudio. El principal factor de confusión identificado para la interpretación de los resultados está relacionado con la granulometría del sedimento, ya que se ha encontrado un efecto directo del contenido en finos de la muestra sobre la toxicidad registrada (Benton *et al.* 1995, Ringwood *et al.* 1997). El más reciente de los protocolos desarrollados por el proveedor del aparato (AZUR 1998a) para muestras sólidas y de sedimento es el Basic Solid Phase Test (BSPT). Este protocolo, más simple y rápido que el SPT, suprime la filtración y mide la emisión de luz cuando la bacteria está aún en contacto con el sedimento, por lo que la emisión de luz no se ve afectada por las bajas tasas de recuperación de las bacterias durante ese paso adicional. El BSPT se ha usado recientemente en distintos estudios (Mowart and Bundy 2001, Abbondanzi *et al.* 2004, Campisi *et al.* 2005) y su uso para la evaluación inicial de toxicidad parece igualmente recomendable.

Material y métodos

Fase I

La Fase I del ejercicio para el ensayo Microtox® SPT se desarrolló sobre seis muestras de sedimento de puertos españoles distribuidas por la Universidad de Cádiz (Casado-Martínez *et al.* 2006) y según el procedimiento operacional estándar (AZUR 1998b). Los parámetros y las condiciones para el desarrollo del ensayo se incluyen en la tabla 1. Cualquier variación al protocolo inicial se incluyó en el informe final de resultados enviado por cada laboratorio participante. Los resultados del

NRRL B-11177 (formerly referred to as *Photobacterium phosphoreum*). Light is emitted as a result of a metabolic pathway linked to the cellular respiration, so disruption of normal cellular respiration produces a rapid decrease in light emission. This assay was developed initially for measuring acute toxicity of natural and waste waters and extracted samples (Bulich 1979), and the basic test has been applied to assess sediment toxicity by exposing bacteria to elutriates, extracted pore water and organic solvent extracts. Although this approach has been widely used for sediment characterization (Demuth *et al.* 1993, Carr *et al.* 1996), more recently it was adapted as a solid-phase test, incorporating the direct contact of bacteria with the whole sediment, its particles as well as pore water (Microbics 1992).

The direct-contact assay developed by AZUR Environmental (Carlsbad, CA, USA) is referred to as the Microtox® solid-phase test (SPT) and over the past few years it has been used to screen for sediment acute toxicity in different surveys (Stronkhorst 2003, Riba *et al.* 2004). The SPT measures the light emission of the bacteria that are recovered by filtration after incubation in direct contact with a slurry of whole sediment and diluent medium. The principal factor affecting the results is the sediment granulometry (Benton *et al.* 1995, Ringwood *et al.* 1997). The basic solid-phase test (BSPT), a new Microtox® test for solid samples, was developed by the manufacturer (AZUR 1998a). This protocol, simpler and faster than the SPT, avoids the filtration step and light emission is measured when the bacteria are still in contact with the sediment sample; hence, the light emission measured is not affected by the low recoveries due to the filtration step. The BSPT has recently been used in different studies (Mowart and Bundy 2001, Abbondanzi *et al.* 2004, Campisi *et al.* 2005) and it seems recommendable for sediment toxicity screening.

Material and methods

Phase I

The Microtox® SPT was performed on sediment samples from Spanish ports distributed by the coordinator laboratory (Casado-Martínez *et al.* 2006). Participating laboratories were asked to perform the test following their own protocol or the protocol described in the literature (AZUR 1998b) sent with the samples (table 1). Participants determined the sample toxicity and were asked to report the results as an IC50 (mg L^{-1}) value, equivalent to the calculated test concentration that inhibits 50% of luminescence relative to controls. The IC50 value was derived using the software supplied with the Microtox® Model 500 Analyzer. In addition, each laboratory reported the wet-weight to dry-weight correction and any change to the initial protocol.

Phase II

A second series of sediment samples was distributed in 2004 by the coordinator laboratory (Casado-Martínez *et al.*

Tabla 1. Parámetros y condiciones a seguir para el desarrollo del SPT con la bacteria marina *Vibrio fischeri*.
Table 1. Test parameters and conditions to develop the solid-phase test (SPT) using the marine bacteria *Vibrio fischeri*.

Facilities and equipment	Photometer (Microtox® Model 500 Analyzer) reading light output at 490 ± 100 nm; incubator for single cuvette at 5.5 ± 1ºC; controlled-temperature room or incubator at 15 ± 0.5ºC
Reconstitution solution	Pure, nontoxic water
Control/dilution water	Diluent purchased from the same commercial supplier or 3.5% NaCl solution
Test temperature	15 ± 0.5ºC
Sample pH, salinity and color/turbidity	No adjustment or correction
Aeration	None required
Subsamples for moisture content	Three replicates of 5 ± 0.2 g dried at 100 ± 0.5ºC for 24 h
Primary dilution	7.00 ± 0.05 g whole, homogenized sediment in 35 mL dilution water in a beaker, mixed for 10 min on a magnetic stirrer, at a rate such that the vortex depth is half the height of the liquid level
Test concentrations	Maximum test concentrations normally 197,000 mg L^{-1} (19.7%, wet wt:vol) on wet-weight basis with two-fold dilutions, for a total of 12 test concentrations in disposable polystyrene tubes; four control solutions; left for 10 min to equilibrate to the test temperature
Test species	*Vibrio fischeri* strain NRRL B-11177, reconstituted by swirling vial three to four times, and mixed ten times with 0.5 mL pipette and held at 5.5 ± 1ºC for 30 min
Inoculum	20 µL into each test concentration, mixed three times with 1.5 mL pipette.
Incubation	20 min at test temperature, filter columns inserted into top of SPT tubes above surface of test concentrations
Filtrate transfer	500 µL into disposable glass cuvettes at test temperature
Observations	Light levels of all test filtrates and controls measured
Endpoint	IC50 (mg L^{-1}), calculated by software; normalized for moisture content of sediment
Reference toxicant	Performed within one month of each test, using suitable positive control sediment and the procedures and conditions for measuring the toxicity of test sediment

ejercicio se recibieron en forma de un valor IC50 (mg L^{-1}), equivalente a la concentración que inhibe el 50% de la luminiscencia con relación a los controles. Para el cálculo de este valor se utilizó el programa informático suministrado con el aparato (Microtox® modelo 500). Cada uno de los laboratorios incluyó también con el informe de resultados los cálculos para la expresión respecto al porcentaje de peso seco de los sedimentos.

Fase II

En 2004, el laboratorio coordinador distribuyó una segunda serie de muestras (Casado-Martínez *et al.* 2006) sobre la que se desarrolló el SPT de acuerdo con el procedimiento operacional estándar. Tres de los laboratorios realizaron el ensayo siguiendo el protocolo BSPT sobre las mismas muestras. El BSPT se desarrolló de acuerdo con el protocolo operacional estándar proporcionado por la casa comercial (AZUR 1998a). Los parámetros y las condiciones para su desarrollo se incluyen en la tabla 2. Aunque se registraron los valores a los 5, 15 y 30 min de incubación sólo se han incluido los resultados de IC50 a los 30 min.

En la tabla 3 se incluyen los laboratorios participantes en cada una de las dos fases y las fechas en las que se desarrolló el 2006). The SPT bioassay was developed according to standard protocol as in Phase I. Three of the laboratories (laboratories 2, 3 and 7) tested the set of samples following the BSPT protocol (AZUR 1998a). Parameters and conditions for the assay and differences between the SPT and BSPT are included in table 2. Although light emission is measured after 5, 15 and 30 min of incubation, only the IC50 results at 30 min were reported. The software supplied with the Microtox® Model 500 Analyzer was used to derive this value.

Data analysis

Interlaboratory precision was estimated by the coefficient of variation (CV) calculated for each sample by dividing the standard deviation (SD) by the mean of the laboratories (X):

$$CV\ (\%) = \frac{SD}{X} \cdot 100$$

This value was used to study interlaboratory precision because it is a very descriptive measure and highly comparable with CVs used in analytical chemistry (USEPA 1994). Nevertheless, it should not be the only approach to evaluate test precision, since CVs may reach very high values when testing

Tabla 2. Parámetros y condiciones a seguir para el desarrollo del BSPT con la bacteria marina *Vibrio fischeri*. Las diferencias con el SPT se marcan con un asterisco.
Table 2. Test parameters and conditions to develop the basic solid-phase test (BSPT) using the marine bacteria *Vibrio fischeri*. Differences between SPT and BSPT are marked with an asterisk.

Facilities and equipment	Photometer (Microtox® Model 500 Analyzer) reading light output at 490 ± 100 nm; incubator for single cuvette at 5.5 ± 1ºC
Reconstitution solution	Pure, nontoxic water
Control/dilution water	Diluent purchased from the same commercial supplier or 3.5% NaCl solution
Test temperature	15 ± 0.5ºC
Sample pH, salinity and color/turbidity	No adjustment or correction
Aeration	None required
Subsamples for moisture content	Three replicates of 5 ± 0.2 g dried at 100 ± 0.5ºC for 24 h
Primary dilution	7.00 ± 0.05 g whole, homogenized sediment in 35 mL dilution water in a beaker, mixed for 10 min on a magnetic stirrer, at a rate such that the vortex depth is half the height of the liquid level
Test concentrations*	Maximum test concentrations normally 99,000 mg L^{-1} on wet-weight basis with two-fold dilutions, for a total of 12 test concentrations; two control solutions; left for 10 min to equilibrate to the test temperature
Test species	*Vibrio fischeri* strain NRRL B-11177, reconstituted by swirling vial three to four times, and mixed ten times with 0.5 mL pipette and held at 5.5 ± 1ºC
Inoculum*	10 µL into each test concentration, mixed three times with 1.5 mL pipette
Incubation*	500 µL into disposable glass cuvettes at test temperature
Observations	Light levels of all test concentrations and controls measured
Endpoint	IC50 (mg L^{-1}), calculated by software; normalized for moisture content of sediment
Reference toxicant	Performed within one month of each test, using suitable positive control sediment and the procedures and conditions for measuring the toxicity of test sediment

ensayo. Los laboratorios se identificaron con un número asignado según el orden de realización del ensayo y por lo tanto los números asignados en la Fase II no corresponden al asignado en la Fase I. La Fase I se completó en un plazo de un mes en todos los laboratorios excepto en los laboratorios 6 y 7, donde el ensayo fue realizado posteriormente. La Fase II del ejercicio se completó en menos de 30 días.

Análisis de los datos

Para el estudio de la reproducibilidad interlaboratorio se calcularon los coeficientes de variación (CV) para cada una de las muestras dividiendo la desviación estándar (SD) por la media de los laboratorios (X),

$$\text{CV (\%)} = \frac{\text{SD}}{\text{X}} \cdot 100$$

Se utilizó este valor para esta estimación de la variabilidad ya que ofrece una medida muy descriptiva y altamente comparable con los CV utilizados en las técnicas para análisis químicos (USEPA 1994). En cualquier caso el CV no debería ser el único método para evaluar la precisión de un ensayo ya que los CVs pueden ser muy altos cuando se estudian muestras extremely toxic or nontoxic samples. In these cases the CV can be higher than 100% but the results can be within an acceptable range. The upper and lower warning limits (UWL = X + 2 · SD and LWL = X – 2 · SD, respectively) were calculated for each sample to avoid subjectivity in the rejection of outliers.

Results

Table 3 summarizes the phase and storage time of the samples before testing at each participating laboratory. A code number was given to each laboratory according to the test date they reported, thus the codes for Phase I are not equivalent to those assigned during Phase II. The results reported by each laboratory for Phases I and II were not compared since it was not the purpose of the exercise. Phase I was completed in one month by all the laboratories except for 6 and 7, which reported results two months later. Phase II was completed successfully in less than one month.

The results of the exercise are summarized in figure 1. The IC50 value of 1000 mg L^{-1} dry weight (Environment Canada 2002) for the SPT was included as reference value to classify the samples according to toxicity results. All samples tested in Phase I (fig. 1a) showed IC50s failing this guideline except for sample A, considered not toxic by all the laboratories even

muy tóxicas o con una toxicidad muy baja. En estos casos el CV puede ser superior al 100% pero las respuestas pueden estar dentro de un rango aceptable. Se calcularon también los valores máximos y mínimos (UWL = X + 2 · SD y LWL = X – 2 · SD, respectivamente) para las muestras analizadas para evitar la subjetividad a la hora de descartar los resultados anómalos.

Resultados

La tabla 3 resume la fase y el tiempo de almacenamiento de las muestras previamente al desarrollo del ensayo para cada laboratorio participante. Un código numérico se ha asignado a cada laboratorio de acuerdo a la fecha de ensayo, es decir, los códigos asignados a los laboratorios en la Fase I no tienen por qué corresponder a los asignados en la Fase II. Los resultados enviados por cada laboratorio para ambas fases no se han comparado entre sí ya que quedaba fuera de los objetivos de este estudio. La Fase I se completó en un mes por todos los laboratorios excepto los laboratorios 6 y 7, que enviaron resultados de ensayos realizados tras más de 8 semanas de almacenamiento de los sedimentos. Los resultados para ambas fases del estudio se resumen en la figura 1. Como valor de referencia para la clasificación de las muestras como tóxicas o no tóxicas (principal objetivo en este tipo de ensayos) se utilizó un IC50 de 1000 mg L^{-1} expresados en peso seco de sedimento para el SPT (Environment Canada 2002). Todas las muestras de la Fase I (fig. 1a) dieron resultados por debajo de este valor excepto la muestra A, la cual fue clasificada como no tóxica por todos los laboratorios aunque con valores de IC50 muy variables. Cada una de las otras cinco muestras fue clasificada como no tóxica por al menos uno de los laboratorios: la muestra E por el laboratorio 4, las muestras B, C y F por el if the IC50 values are different. The rest of the samples were classified as not toxic by at least one laboratory: laboratory 4 classified sample E as not toxic; laboratory 6, samples B, C and F; and laboratory 7, sample D. Samples tested during Phase II (fig. 1b) were classified more homogeneously except for samples D and E: sample D was considered not toxic by all

Tabla 3. Laboratorios participantes en cada fase del estudio y días después de la recogida de las muestras en que se realizó el ensayo.
Table 3. Laboratories participating in each study phase and days after sediment sampling when tests were performed.

	Laboratory	Days after sediment sampling	Test
Phase I	1	14 days	SPT Phase I
	2	29 days	SPT Phase I
	3	30 days	SPT Phase I
	4	35 days	SPT Phase I
	5	45 days	SPT Phase I
	6	100 days	SPT Phase I
	7	–	SPT Phase I
Phase II	1	3 days	SPT Phase II
	2	8 days	SPT Phase II and BSPT
	3	8 days	SPT Phase II and BSPT
	4	11 days	SPT Phase II
	5	16 days	SPT Phase II
	6	18 days	SPT Phase II
	7	29 days	SPT Phase II and BSPT

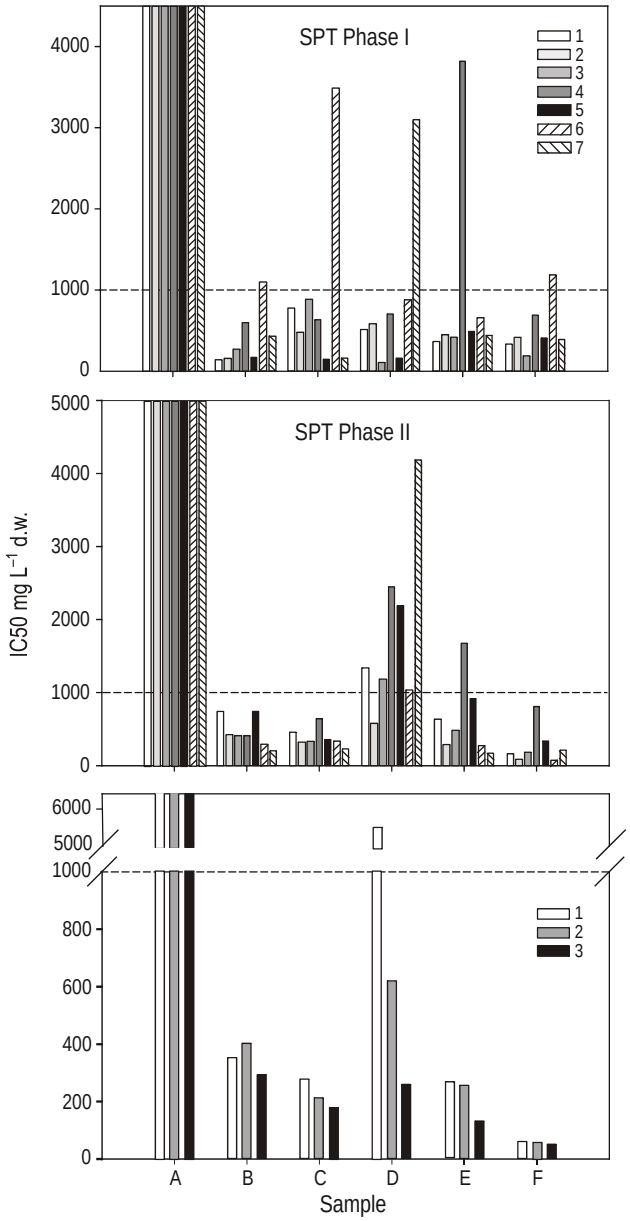

Figura 1. Resultados del bioensayo con *Vibrio fischeri* durante el ejercicio de comparación interlaboratorio. (a) SPT Fase I, (b) SPT Fase II y (c) BSPT Fase II. El valor de 1000 mg L^{-1} en peso seco canadiense está incluido como valor de toxicidad de referencia.
Figure 1. Results of the *Vibrio fischeri* bioassays during the interlaboratory comparison. (a) Microtox® solid-phase test (SPT) Phase I, (b) SPT Phase II and (c) basic solid-phase test (BSPT) Phase II. The Canadian benchmark of 1000 mg L^{-1} dry weight is included as the reference toxicity value.

laboratorio 6, y la muestra D por el laboratorio 7. Las muestras ensayadas durante la Fase II (fig. 1b) resultaron clasificadas de manera muy similar, a excepción de las muestras D y E que fueron clasificadas como no tóxica y tóxica, respectivamente, por todos los laboratorios menos uno en cada caso. El protocolo BSPT (fig. 1c) mostró resultados similares en todos los laboratorios participantes excepto para la muestra D que mostró una toxicidad muy distinta en uno de los laboratorios. Dado que no se encontró ningún otro factor de confusión durante el ensayo es posible que esta diferencia fuese debida a la homogeneización y/o muestreo de los sedimentos a la hora de hacer el reparto.

En las tablas 4, 5 y 6 se presentan los resultados de cada laboratorio y el promedio, la desviación estándar, el coeficiente de variación, y los valores máximos y mínimos de aceptación de los resultados calculados para cada muestra. Para la Fase I estos valores se calcularon para dos series de datos: una incluyendo los resultados de todos los laboratorios y otra sin incluir aquellos laboratorios que hicieron el ensayo fuera de los límites de tiempo (es decir, excluyendo los resultados de los laboratorios 6 y 7) y excluyendo los datos fuera de los rangos de aceptabilidad (UWL y LWL). Debido al bajo número de casos (laboratorios) los rangos de aceptabilidad de los resultados eran muy amplios, pero en total tres datos se encontraron fuera de ellos: dos datos correspondientes a ensayos realizados después del periodo de conservación de los sedimentos (cuatro

the laboratories except for 2, while sample E failed the toxicity guideline in all cases except for laboratory 4, which reported a higher value. The BSPT results (fig. 1c) were similar for all the laboratories except in the case of sample D, which had different toxicity values.

The IC50 values reported by each laboratory and the X, SD, CV, UWL and LWL are presented in tables 4, 5 and 6. Statistics were performed for two different data sets: one including the results of all the laboratories and the other including only the results of the laboratories that tested the samples in the four weeks after the sampling (i.e., excluding laboratories 6 and 7) and excluding the outliers. The few laboratories involved in the exercise produced very wide UWL and LWL. Nevertheless, three values failed the UWL and were rejected: two obtained after the four-week period and one reported by laboratory 4. The CV varied between 64% and 134%, with a mean CV of 103% when all the laboratories were included. When only the test results of the laboratories that performed the test in the four weeks following the sampling were included in the calculation, the CV ranged from 45% to 75%, with a mean CV of 53%.

The Phase II results classified sample A' as not toxic. Sample C' had a mean IC50 value near 1000 mg L^{-1} dry weight and the other samples, 500 mg L^{-1} dry weight or lower. Only one value, reported by laboratory 4, was rejected. The CV ranged

Tabla 4. Valores estadísticos calculados para cada muestra (A–F) de la Fase I del estudio. X', SD', CV', UWL' y LWL' se han calculado para los laboratorios que cumplieron los límites establecidos para el tiempo de conservación de las muestras.
Table 4. Statistical values calculated for each sample (A–F) in Phase I of the study. The mean (X'), standard deviation (SD'), coefficient of variation (CV'), upper warning limit (UWL') and lower warning limit (LWL') were calculated for those laboratories that complied with the limits established for the storage period of the samples.

	A	B	C	D	E	F
Laboratory 1	29026	131.6	774.5	506.6	357.81	330.97
Laboratory 2	34766	147.67	476.89	571.66	442.34	408.42
Laboratory 3	4531	263	880	99	418	184
Laboratory 4	41543	596.7	630	703	3806*	693
Laboratory 5	4748	165	143	158	487	404
Laboratory 6*	11810	1090	3470	870	650	1180
Laboratory 7*	82580	429.96	170.92	3102.7	436.99	390.59
X	29857.71	403.42	935.04	858.71	942.59	513.00
SD	27503.42	347.69	1152.46	1027.49	1265.92	330.77
CV	92.11	86.18	123.25	119.66	134.30	64.48
UWL	84864.55	1098.79	3239.96	2913.69	3474.43	1174.54
LWL	–25149.12	–291.95	–1369.88	–1196.27	–1589.25	–148.55
X'	22922.80	260.79	580.88	407.65	426.29	404.08
SD'	17268.50	194.62	288.07	265.29	53.86	185.25
CV'	75.33	74.62	49.59	65.08	12.63	45.85
UWL'	57459.79	650.03	1157.01	938.23	534.00	774.58
LWL'	–11614.19	–128.44	4.75	–122.92	318.57	33.57

* Values not included in the calculation of the statistical parameters.

Tabla 5. Valores estadísticos calculados para cada muestra (A–F) de la Fase II del estudio. Resultados para el SPT.
Table 5. Statistical values calculated for each sample (A–F) of the solid-phase test in Phase II of the study.

	A	B	C	D	E	F
Laboratory 1	74500	750	450	1330	630	160
Laboratory 2	20255	421	317	573	289	78
Laboratory 3	72533.8	407.93	330.32	1176.03	479.57	177.1
Laboratory 4	Not toxic	414.6	647.8	2446	1677	811.5*
Laboratory 5	242603	748	360.4	2190.4	916.7	343.1
Laboratory 6	9146	283	330	1028	261	68
Laboratory 7	7174.75	209.56	235.4	4185.67	172.43	221.57
X	71035.43	462.01	381.56	1847.01	632.24	101.50
SD	89372.05	211.09	133.44	1221.84	526.44	174.63
CV%	125.81	45.69	34.97	66.15	83.26	58.13
UWL	249779.53	884.19	648.44	4290.69	1685.12	781.47
LWL	–107708.67	39.83	114.68	–596.67	–420.64	–250.25

* Value not included in the calculation of the statistical parameters.

Tabla 6. Valores estadísticos calculados para cada una de las muestras (A–F) de la Fase II del estudio para el BSPT.
Table 6. Statistical values calculated for each sample (A–F) of the basic solid-phase test in Phase II of the study.

	A	B	C	D	E	F
Laboratory 1	31158	349.43	278.64	5872	268.58	60.27
Laboratory 2	Not toxic	402	217	623	258	63
Laboratory 3	12462	294	179	258	131	48.78
X	21810	348.47	224.88	2251	219.19	57.35
SD	13220.07	54.01	50.29	3141.18	76.56	7.55
CV	60.61	15.50	22.36	139.55	34.93	13.16
UWL	48250.14	456.49	325.45	8533.37	372.31	72.44
LWL	–4630.14	240.46	124.31	–4031.37	66.07	42.26

semanas) y un dato proporcionado por el laboratorio 4. Estos datos, que en todos los casos correspondieron a una toxicidad menor a la estimada por el resto de los laboratorios, fueron eliminados de los cálculos posteriores. Cuando se incluyen todos los laboratorios, los CV iban de 64% a 134% con un promedio de 103%. Cuando se excluyen los laboratorios que hicieron sus ensayos a destiempo estos valores se encuentran entre 45% y 75% con un promedio de 53%.

En la Fase II la muestra A' fue clasificada claramente como no tóxica pero igual que en la Fase I con valores de IC50 muy distintos. La muestra C' dió valores alrededor de 1000 mg L^{-1} y las otras muestras resultaron con valores de 500 mg L^{-1} o menores. Sólo un valor, proveniente del laboratorio 4, estuvo fuera de los UWL y LWL, y fue excluido del resto de los cálculos. Los CV variaron entre 34% para la muestra C' y hasta 125% para la muestra A', con un promedio de 69% (57% si se excluye la muestra A'). Los resultados para el protocolo BSPT fueron más homogéneos aunque el CV para la muestra D fue muy elevado (139%). Para la muestra A, al igual que con el

from 34% for sample C to 125% for sample A, with a mean CV of 69% (58% excluding sample A). The BSPT results classified the samples more homogeneously even though the CV for sample D was very high (139%). For sample A, as for the SPT, results were very variable although the CV was lower than using the SPT (60%). The rest of the samples reported CVs between 13% and 22%.

Discussion

Sample A, which was classified in category I according to the chemical characterization (Casado-Martínez *et al.* 2006) and thus suitable for open water disposal after a normal authorization process, was also considered not toxic according to the Microtox® bioassay and causes the high interlaboratory variability. This sample reported the highest CV, probably related to the sediment particle size. The sediment grain size is an important confounding factor when interpreting Microtox® test results. Even though research has focused on the effects when

SPT, los resultados fueron muy variables aunque el valor de CV disminuye hasta 60%. Para el resto de muestras el CV varió entre 13% y 22%.

Discusión

Al comparar los resultados de la caracterización química de los sedimentos (Casado-Martínez *et al.* 2006) con los resultados del ensayo de toxicidad para la muestra A, que había sido clasificada en la categoría I y por tanto podría ser considerada apta para su vertido al mar tras una autorización normal, ésta también se clasifica claramente como no tóxica y esto causa la variabilidad de los valores obtenidos, pero con un alto CV. Como se ha mencionado anteriormente, la distribución del tamaño de los sedimentos se ha identificado como un importante factor de confusión a la hora de interpretar los resultados de este ensayo. El interés general se ha centrado en identificar la medida del efecto de la presencia de sedimentos finos en la toxicidad, habiéndose propuesto distintas correcciones (Ringwood *et al.* 1997, Stronkhorst 2003) aunque no parecen recomendables para la gestión de dragados en puertos españoles (DelValls *et al.* 2003). En el caso de muestras con porcentajes de arenas elevados, el efecto del tamaño de grano incrementa directamente la variabilidad del ensayo ya que existen dificultades para resuspender efectivamente el sedimento. Esta dificultad resulta especialmente decisiva a la hora de coger la alícuota de la suspensión inicial con lo que las concentraciones teóricas no se corresponden con las concentraciones reales a las que las muestras están expuestas y, por lo tanto, el dato de IC50 no es representativo de la toxicidad real de la muestra. En este sentido la agencia ambiental canadiense, que utiliza un valor absoluto para la clasificación de la toxicidad de los sedimentos, reconoce la problemática para la clasificación de las muestras arenosas y, si el sedimento en cuestión contiene un porcentaje de finos (igual o menos a 0.063 mm) mayor que 20% recomienda el uso de un sedimento control negativo de toxicidad con el cual comparar los resultados del sedimento problema (Environment Canada 2002); no obstante, la variabilidad entre los replicados continúa siendo un factor importante que debe tenerse en cuenta si los resultados van a ser utilizados para la autorización de permisos y la vigilancia ambiental. Otro factor a tener en cuenta pero que puede ser fácilmente minimizado es el tiempo de almacenamiento de los sedimentos. Las guías disponibles para la realización de ensayos de toxicidad en el laboratorio ya incluyen tiempos máximos dentro de los cuales deben de realizarse los ensayos de toxicidad para que los resultados sean representativos ya que durante el periodo de conservación de los sedimentos las especies químicas pueden variar en cantidad o en su forma disponible (GIPME 2000). Aunque el efecto de este factor puede ser más o menos evidente según la importancia de otros factores de confusión, se ha podido identificar cierta tendencia a generar valores superiores de IC50 y por lo tanto a subestimar la toxicidad de los sedimentos. Aunque este factor puede no tener mayor importancia cuando se estudian muestras no testing fine-grained sediments and different corrections have been proposed (Ringwood *et al.* 1997, Stronkhorst 2003), they do not seem useful for dredged material characterization in Spain (DelValls *et al.* 2003). When testing sediment samples with a high percentage of sand a high variability in the results is expected because it is difficult to make the sediment suspension for the primary dilution. This is especially important since the theoretical test concentrations can be very different from the actual concentration to which the bacteria are exposed and the IC50 value would not be representative of sediment toxicity. The Canadian agency uses an absolute value for sediment classification and recognizes the differences when testing these kinds of samples. It recommends the use of a sediment with similar characteristics as a negative toxicity control to which results must be compared (Environment Canada 2002) when the percentage of fine sediments (≤0.063 mm) is lower than 20%. Nevertheless, the variability is not solved in this way and it is still an important factor to consider if the results are used for regulatory or environmental monitoring purposes.

Another confounding factor to consider is the sediment storage period, though this can be easily avoided. Since sediment chemical species can change in quantity and availability during the storage period (GIPME 2000), protocols for sediment toxicity already include maximum storage guidelines in order to obtain representative results. In this study the influence of time storage was evident for those laboratories that reported results later in time with unrandom tendencies in higher IC50 values. Although this factor may not be of special interest when testing noncontaminated samples, it seems that it can have a great influence on the classification of organic contaminated sediments, such as samples B and D that also reported the highest CVs.

The CVs for Phase II were similar to those found in Phase I but sample classification seems more homogeneous in Phase II. The reduction of the sample storage period or the greater experience acquired by the laboratories between both phases could have reduced this variability. The BSPT results seem to be of special interest with the lowest CVs, possibly because of the small number of interfering factors. The BSPT methodology avoids the filtration step, so there is no effect on the luminescence due to poor recoveries of the bacteria. Even though this protocol does not include the possibility of performing the assay on two replicates simultaneously, such as the SPT, the percentage of inhibition is calculated comparing the light emission after incubation in contact with the sample to the initial light emission from the same test dilution before the addition of the sediment. Since the SPT uses two control blanks to calculate the percentage of inhibition, the BSPT avoids the variability related to the volume of bacterial suspension added to each test dilution. According to our results and the confounding factors for their interpretation, Microtox® seems suitable to be used as a screening tool for dredged material toxicity characterization in Spain. It would be desirable to establish interim pass-fail criteria such as those

contaminadas, sí puede afectar decisivamente cuando se trata de sedimentos con contaminación de tipo orgánico, como es el caso de los sedimentos B y D que presentan una mayor variabilidad.

Los coeficientes de variación calculados en la Fase II del ejercicio son semejantes a los encontrados durante la Fase I, pero los resultados al clasificar las muestras parecen más homogéneos. Es posible que esto se deba a que el segundo ensayo se realizó en todos los laboratorios dentro del primer mes después de la recogida de los sedimentos, aunque también es de esperar una mayor experiencia de los laboratorios ya para la Fase II. De especial interés son los resultados para el protocolo BSPT en el que se obtuvieron CVs más bajos. El BSPT tiene dos ventajas importantes respecto al SPT que pueden contribuir en la reducción de la variabilidad interlaboratorio. La primera de ellas es la supresión de la filtración de la muestra, que evita el efecto en la disminución de la luminiscencia debido a la pobre recuperación de las bacterias del sedimento. Aunque el BSPT no incluye la posibilidad de incorporar un duplicado de la muestra como en el SPT, el porcentaje de inhibición de la luminiscencia se calcula para cada concentración analizada respecto al valor de emisión de las bacterias medido para cada concentración previamente a la adición de las concentraciones del sedimento problema y no respecto al valor de cuatro blancos como sucede con el SPT. De este modo se evita la variabilidad introducida por el volumen de reactivo (bacterias) adicionado a cada concentración, el cual puede ser muy distinto dependiendo de la experiencia del operador de la pipeta. La baja variabilidad interlaboratorio registrada para el protocolo BSPT también puede deberse a la mayor experiencia de los laboratorios con el ensayo aunque no todos habían utilizado este nuevo protocolo de forma rutinaria.

Según los resultados de este ejercicio, y teniendo en cuenta los factores de confusión en la interpretación de los resultados, el ensayo Microtox® parece adecuado como herramienta para la caracterización inicial de la toxicidad de materiales de dragado en España. En este sentido se recomienda el establecimiento de un valor guía como los utilizados en Holanda o Canadá de alrededor de 1000 mg L^{-1} expresados en peso seco de sedimento. La comparación entre el antiguo SPT y el nuevo protocolo BSPT parece apuntar a este último como el más recomendable no sólo por resultados previos con muestras de sedimentos (Campisi *et al.* 2005), sino también porque su diseño es más simple y económico en términos de tiempo y esfuerzo, y dada su menor variabilidad interlaboratorio, pudiera también ser más recomendable para efectos regulatorios. La disminución en los CV con el protocolo BSPT puede ser debida a una mayor experiencia en el desarrollo del ensayo o bien a la menor variabilidad en la metodología. En cualquier caso, la variabilidad sigue siendo elevada para muestras con alto porcentaje de arenas.

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Nota de Investigación/Research Note

Ejercicio interlaboratorio con bioensayos marinos para la evaluación de la calidad ambiental de sedimentos costeros. III. Bioensayo con embriones del erizo de mar *Paracentrotus lividus*

Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. III. Bioassay using embryos of the sea urchin *Paracentrotus lividus*

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Resumen

El presente trabajo resume los resultados del ejercicio realizado para estudiar la variabilidad interlaboratorio del ensayo con estadios larvarios del erizo de mar *Paracentrotus lividus*. Este ejercicio, que se desarrolló en dos fases distintas, incluyó cuatro laboratorios cada uno de los cuales estudió la toxicidad de las seis muestras de sedimento distribuidas. Las muestras, provenientes de distintos puertos de la costa española, se caracterizaron mediante la exposición de embriones del erizo de mar *Paracentrotus lividus* durante 48 h a los lixiviados de los sedimentos. La Fase I se utilizó para rediseñar las condiciones del ensayo y evitar posibles factores de confusión al interpretar los resultados. Los resultados de la Fase II fueron más homogéneos al clasificar las muestras según la toxicidad registrada, a pesar de la variabilidad en los protocolos de obtención y ensayo de los lixiviados. De acuerdo con estos resultados, el ensayo es adecuado para la caracterización de este tipo de muestras con una variabilidad interlaboratorio similar a la encontrada para otros bioensayos en estudios interlaboratorio previos.

Palabras clave: material de dragado, toxicidad embrionaria, ecotoxicología, lixiviados.

Abstract

The present paper reports the results of an interlaboratory variability study of a bioassay using larval stages of the marine sea urchin *Paracentrotus lividus*. This exercise was developed in two different phases and included four laboratories, each of which determined the toxicity of six sediment samples. The samples were collected from different Spanish ports and were characterized by exposing sea urchin embryos for 48 h to sediment elutriates. Phase I was used to redesign test parameters and to avoid possible interfering factors when interpreting test results. Laboratories were more homogeneous in the classification of sediments according to the toxic responses in Phase II despite the high variability of the elutriate testing protocols. Based on our results, the test seems suitable to characterize dredged material, the interlaboratory variability being similar to that found for other bioassays in previous studies.

Key words: dredged material, embryo toxicity, ecotoxicology, elutriates.

Introducción

Los bioensayos con estadios embrionarios y larvarios de invertebrados marinos han sido frecuentemente utilizados para evaluar la calidad ambiental de muestras de sedimentos (Carr 1996; DelValls *et al.* 1998; Beiras *et al.* 2001, 2003a, 2003b; Mariño-Balsa *et al.* 2003), así como la toxicidad de contaminantes concretos (Fernández y Beiras 2001, Cesar *et*

Introduction

Bioassays using marine invertebrate embryos and larvae have been widely used to evaluate environmental quality (Carr 1996; DelValls *et al.* 1998; Beiras *et al.* 2001, 2003a, 2003b; Mariño-Balsa *et al.* 2003) and the toxicity of contaminants (Fernández and Beiras 2001, César *et al.* 2002). Moreover, they have been considered a rapid and sensitive tool to

al. 2002), y son considerados un método rápido y sensible para la caracterización de la toxicidad de sedimentos marinos. Entre los bionsayos embrio-larvarios más utilizados se encuentran los realizados con ostras (*Crassostrea gigas)* y con erizos de mar (*Paracentrotus lividus*, *Sphaerechinus granularis*, *Strongylocentrotus purpuratus*, *S. droebachiensis*, *Dendraster excentricus* o *Arbacia punctulata*). Estas especies son abundantes y se encuentran distribuidas a lo largo de las costas españolas, generalmente son fáciles de recoger y pueden ser mantenidas fácilmente en el laboratorio. La obtención de gametos y su fecundación *in vitro* son relativamente simples y, debido a la rapidez con que se completa el desarrollo embrionario, pueden obtenerse resultados en un corto periodo de tiempo. Desde que el erizo de mar fue utilizado por primera vez en 1951 por Wilson, se han desarrollado numerosas investigaciones para la estandarización de protocolos y para evitar los diferentes factores de confusión que pueden interferir en los resultados y su interpretación. Hoy en día existen distintos protocolos estándar (SOPs) para la evaluación de la toxicidad de lixiviados de sedimentos con distintas especies, entre ellos cabe citar el USEPA (1995) para *S. purpuratus* y *S. droebachiensis*, RIKZ (1999) para *C. gigas* y el de la ASTM (1995) para *A. punctulata*, *S. droebachiensis*, *S. purpuratus* y *D. excentricus.*

Para este ejercicio interlaboratorio se distribuyó a cada laboratorio participante un protocolo obtenido de acuerdo con los distintos estándares disponibles. Los principales factores que podían contribuir a la variabilidad interlaboratorio incluían el distinto origen de los organismos (y por lo tanto distinto estado de desarrollo gonadal, distinta aclimatación y manipulación) y la introducción de variantes durante el proceso de obtención de los lixiviados. Otras fuentes de variabilidad importantes están relacionadas al contenido en sulfuros o material en suspensión de los lixiviados (Fernández 2002). El objetivo de este trabajo fue evaluar la habilidad de los diferentes laboratorios para caracterizar materiales de dragado mediante el ensayo con el desarrollo embrionario de erizos de mar, y evaluar las diferencias obtenidas entre los resultados de cada laboratorio.

Material y métodos

Fase I

El primer ejercicio de intercalibración se llevó a cabo en 2003. En esta primera fase se evaluó la toxicidad de seis muestras de sedimento provenientes de distintas zonas de la costa española (Casado-Martínez *et al.* 2006). Para la obtención de los lixiviados se recomendó la siguiente modificación del método USEPA (1998): los sedimentos previamente homogeneizados debían mezclarse con agua de mar control en una proporción 1:4 v/v (sedimento:agua) y mantenerse en agitación rotatoria durante 30 min a 20ºC. Se recomendó un tiempo de decantación de 12 h transcurrido el cual se retiraría el sobrenadante sin remover el sedimento del fondo.

characterize marine sediment toxicity. The most common bioassays are those using the oyster *Crassostrea gigas* and the sea urchins *Paracentrotus lividus*, *Sphaerechinus granularis*, *Strongylocentrotus purpuratus*, *S. droebachiensis*, *Dendraster excentricus* and *Arbacia punctulata*. These species are abundant and widely distributed along the coast of Spain, they are easy to collect and can be maintained in the laboratory for long periods of time. Gametes and fecundation are easy to obtain and since the embryo develops rapidly the larval phase can be completed in a short period. The sea urchin test was first used in 1951 and since then much research has been conducted to standardize the protocol and to avoid the different interfering factors affecting data interpretation. Diverse standard operational procedures (SOPs) to assess sediment elutriate toxicity using different species are available from several agencies, among them those of USEPA (1995) for *S. purpuratus* and *S. droebachiensis*, of RIKZ (1999) for *C. gigas*, and of ASTM (1995) for *A. punctulata*, *S. droebachiensis*, *S. purpuratus* and *D. excentricus.*

A protocol based on available SOPs was sent to the laboratories participating in this exercise even if all of them had previous experience using this test. Variability factors, discussed further in the results section, included different batches of organisms (different gonad development, acclimation and handling) and different elutriation procedures. Other important sources of variability were the possible seasonal viability of eggs and the presence of hydrogen sulphide or suspended solids in elutriates (Fernández 2002). The main objective was to evaluate the ability of the different laboratories to characterize dredged materials as toxic or not toxic and to determine interlaboratory variability.

Material and methods

Phase I

The first phase of the study was developed in 2003. Six sediment samples from Spanish ports were sent to each laboratory (Casado-Martínez *et al.* 2006). A modification of the USEPA (1998) method was recommended to obtain the elutriates: previously homogenized sediments were mixed with clean seawater at a ratio of 1:4 v/v (sediment:water) for 30 min at approximately 20ºC. A settling period of 12 h was recommended before the overlying water was siphoned.

Embryos were obtained from a couple of mature organisms of the species *P. lividus*, collected from sites where the laboratories were located. Gametes were obtained either by dissecting the organisms and extracting them directly with a pipette or by osmotic shock (using 1 mL KCl 0.5 N). Eggs were maintained in seawater, while sperm was kept dry and refrigerated until being used. Less than 30 min after obtaining the gametes, optimal eggs were mixed with a few microlitres of sperm in a measuring cylinder with control seawater. After gently stirring the suspension to facilitate fecundation, which was completed in a few minutes, the density and percentage of

Los embriones utilizados para las pruebas provenían de una sola pareja de organismos maduros de *P. lividus*, recogidos *in situ* en la localidad correspondiente a cada laboratorio. Los gametos se obtuvieron o bien provocando la puesta por choque osmótico (inyectando 1 mL de KCl 0.5 M) o mediante la disección de los organismos y la extracción directa de los gametos. Los huevos se mantuvieron en agua de mar control mientras que el esperma se conservó en seco y en frío hasta el momento de su uso. Treinta minutos o menos después de la obtención de los gametos se realizó la fecundación *in vitro* añadiendo unos microlitros de esperma a la suspensión de huevos en agua de mar control. Tras una agitación suave para facilitar la fecundación, que ocurre en pocos minutos, se estimaron la densidad y el porcentaje de fecundación (indicado por la presencia de la membrana de fecundación característica) en al menos tres muestras y se tomó el valor medio. Una vez conseguida la fecundación, se introdujeron 20–30 embriones mL^{-1} en los recipientes de incubación conteniendo los lixiviados. Se recomendó un mínimo de cuatro réplicas por cada uno de los lixiviados, más una serie control conteniendo agua de mar como matriz. Este control negativo de toxicidad permite evaluar la calidad del agua utilizada para la obtención de los lixiviados así como la idoneidad del material biológico, evitando falsos positivos. Pasadas 48 h de incubación a 20ºC y oscuridad, las muestras se fijaron con unas gotas de formaldehído al 40%. La respuesta biológica estudiada fue el éxito en la embriogénesis tras el periodo de incubación, medido como porcentaje de larvas pluteus normales (que presentraron los cuatro brazos bien desarrollados). Esta respuesta fue observada en cada réplica de 100 individuos. Los parámetros y condiciones para el desarrollo del ensayo se recogen en la tabla 1.

Fase II

El segundo ejercicio se realizó en 2004. El ensayo se realizó de acuerdo al protocolo estándar proporcionado para la Fase I, con algunas modificaciones encaminadas a mejorar la homogeneidad metodológica en base a los resultados obtenidos durante la fase previa. Se recomendó la aireación de los lixiviados antes de la incubación de los organismos, con el objetivo de eliminar falsos positivos causados por la presencia de sulfuros o amonio en las muestras.

Análisis de los resultados

Las diferencias significativas entre las respuestas a los distintos lixiviados se determinaron mediante ANOVA y el test de Tukey. Previamente se comprobó la homocedasticidad de los datos aplicando el test de Levene. Estos análisis se realizaron con el software estadístico SPSS 11.5. Para estudiar la variabilidad interlaboratorio se utilizó el coeficiente de la varianza (CV) calculado como el cociente entre la desviación estándar (SD) y la media de todos los laboratorios (X):

$$CV\ (\%) = \frac{SD}{X} \cdot 100$$

fecundation (presence of the fertilization membrane) were observed in at least three samples.

Once fecundation was successfully completed, embryos were introduced in 20-mL vials with the sediment elutriates at 20ºC at a density of 20–30 embryos mL^{-1}. A minimum of four replicates were used per sample and a negative toxicity control consisting of clean seawater (the same used to obtain the elutriates) was tested in parallel with the samples to evaluate the seawater quality and biological material and to avoid any false positive response. After 48 h at 20ºC and darkness, the samples were fixed with two drops of 40% formaldehyde. Laboratories were asked to report the success of embryogenesis after the incubation period, calculated as the percentage of normal pluteus (defined as those with four well-developed arms). For this endpoint, 100 organisms were studied per replicate. Test parameters and conditions are summarized in table 1.

Phase II

A second set of sediment samples was distributed in 2004 (Casado-Martínez *et al.* 2006). The tests followed the protocol used in Phase I, though some modifications were recommended after considering the Phase I results to homogenize the methodology among laboratories. Aerating the elutriates prior to the introduction of embryos was recommended to avoid false positive responses caused by the presence of ammonia or sulphide.

Data analysis

An ANOVA and Tukey's test were used to establish differences among the responses. Levene's test was used to ensure normality and homogeneity of the data. The SPSS 11.5 software was used to develop these tests. To study interlaboratory variation, the coefficient of variation (CV) was calculated by dividing the standard deviation (SD) by the mean of the laboratories (X):

$$CV\ (\%) = \frac{SD}{X} \cdot 100$$

Results

The days elapsed from receiving the sediments to performing the tests in each of the participating laboratories in both phases of the exercise are summarized in table 2. Five laboratories already trained in this bioassay using sea urchin embryos were asked to participate in Phase 1, whereas four laboratories participated in the Phase II of this study. Phase I was successfully completed by all laboratories in less than one month after sediment sampling (USEPA 1994), so differences due to time storage were not expected. Phase II was successfully developed by all laboratories in a few days following sediment sampling, except for laboratory 4 that performed the assay after three months due to logistic problems.

Tabla 1. Parámetros y condiciones para el desarrollo del bioensayo con embriones de erizo de mar recomendados a los laboratorios para la realización de este ejercicio.
Table 1. Parameters and conditions to develop the test using sea urchin embryos in the laboratory.

Parameters	Conditions
Type of test	On elutriate; static
Temperature	20ºC
Salinity	30–40
Photoperiod	16:8 h light:dark or darkness
Test chambers	20 mL
Water renewal	None
Density in test chamber	20–30 embryo mL^{-1}
Number of replicates	4
Aeration	Soft for 5 min before introducing the embryos
Water quality	Temperature, salinity, pH and dissolved oxygen
Test duration	48 h
Endpoints	Embryo success (percentage of normal pluteus larvae)
Number of measures per replicate	100
Test acceptability	90% normal larvae in controls

Resultados

En la tabla 2 se muestran los laboratorios participantes y los días transcurridos desde la recepción de las muestras hasta que se desarrollaron ambas fases del estudio. El ejercicio contó con cinco participantes para la Fase I y cuatro para la Fase II con la estructura necesaria para realizar el bioensayo. Todos ellos finalizaron la primera fase en menos de un mes desde la recepción de los sedimentos (USEPA 1994) y por lo tanto no se esperan diferencias debidas a distintos tiempos de almacenamiento de los sedimentos. Para la Fase II del estudio todos los laboratorios iniciaron el ensayo en menos de tres días desde la recepción de las muestras, excepto el laboratorio 4 que realizó el ensayo después de tres meses de almacenaje de los sedimentos debido a problemas logísticos.

Los resultados de la Fase I se resumen en la tabla 3. Aunque se envió un protocolo con las muestras de sedimento, se encontraron diferencias importantes en las condiciones de ensayo de los distintos laboratorios: en algunos laboratorios los lixiviados se airearon antes de introducir los organismos y/o se filtraron después de la decantación. Todos los factores que podían introducir variabilidad en los resultados fueron estudiados antes de la preparación de la serie de muestras enviadas en la Fase II. Otro factor a tener en cuenta es el resultado de los controles de toxicidad negativos en tres de los laboratorios (marcados con un asterisco en la tabla) ya que no cumplían los criterios de aceptabilidad del ensayo al no obtenerse el porcentaje mínimo de larvas normales del 90%. Esto puede estar indicando una baja calidad del agua o del material biológico detectándose una toxicidad espuria.

Otros factores de confusión importantes son los relacionados con la obtención del lixiviado, como por ejemplo el uso de

Phase I results are summarized in table 3. Even though a protocol was sent with the sediment samples, important differences were reported by the laboratories: some laboratories aerated the elutriates and/or filtered them after settling. All factors were studied before the next sediment samples were sent out with the new protocol in Phase II. Another important factor was the percentage of normal pluteus registered by three laboratories (marked with an asterisk in table 3) for the negative toxicity control since they did not meet the acceptance criterion of 90% normal pluteus. Low fertility values in the controls can be due to poor seawater quality or poor quality of the biological material.

The use of different methods during elutriation, such as employing magnetic stirrers instead of vortex methods or increasing the settling time, may also have caused variability in the results because they can affect the test sensibility by

Tabla 2. Tiempo transcurrido desde la recepción de los sedimentos hasta el desarrollo del bioensayo, en cada uno de los laboratorios participantes en ambas fases del ejercicio. La fecha de ensayo se ha contado desde el día de muestreo de los sedimentos.
Table 2. Time elapsed since the reception of sediments until developing the bioassay at each participating laboratory for both phases of the exercise. Test date is counted from the day of sediment sampling.

Laboratory	Test date Phase I	Test date Phase II
1	1 day	5 days
2	28 days	8 days
3	27 days	8 days
4	8 days	90 days
5	15 days	

Tabla 3. Precisión interlaboratorio de los resultados de mortalidad media del bioensayo de toxicidad con embriones de erizo de mar para la primera fase del ejercicio. n.a. significa dato no disponible.
Table 3. Interlaboratory precision of the mean percentage of normal pluteus from sediment toxicity tests using sea urchin embryos during Phase I; n.a. = not available, X = mean, SD = standard deviation and CV = coefficient of variation.

Laboratory	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Control
1	n.a.	85.2	3.8	96.4	n.a.	n.a.	96.8
2	0.0	0.0	14.7	45.0	50.3	43.7	76.7*
3	68.5	80.0	17.8	89.5	64.6	0.0	81.5*
4	16.5	5.6	0.0	92.0	93.0	94.5	93.8
5	34.5	12.0	10.8	86.6	76.0	87.0	84.7*
X	29.9	36.6	9.4	81.9	71.0	56.3	86.7
SD	29.4	42.3	7.4	20.9	18.0	43.7	8.4
CV %	98.3	116.0	78.8	25.6	25.4	77.7	9.73

* Controls not meeting the acceptance criterion

agitadores magnéticos en lugar del método del volteo, o el aumento del tiempo de decantación de la mezcla. Estos factores pueden influir en el resultado y la sensibilidad del ensayo ya que afectan directamente a la movilidad de los distintos contaminantes desde el sedimento a la fase líquida. Un factor relacionado con éstos es la aireación de la muestra. En este sentido se ha registrado una extremadamente alta inhibición en el desarrollo embrionario con la muestra A, con niveles de contaminación, aunque muy bajos, según los resultados de los análisis químicos (Casado-Martínez *et al.* 2006) y que podrían estar directamente relacionados con la presencia de sulfuro de hidrógeno. Esta sustancia, presente de forma natural en los sedimentos anóxicos y cuya concentración puede aumentar durante el almacenamiento de la muestra, puede producir resultados de toxicidad elevada para el ensayo con embriones del erizo de mar que pueden ser atribuidos erróneamente a la presencia de contaminantes en el sedimento (Lapota *et al.* 2000). En este sentido, al parecer, las muestras que han podido verse afectadas por este factor son las correspondientes al puerto de Cádiz (muestras A y B) con una gran variabilidad en el porcentaje de larvas normales y en la clasificación de las muestras. El CV medio para la Fase I fue de 61%, con valores comprendidos entre 9.7% para el control de toxicidad negativo y 115% para la muestra B. Las muestras D y E, no tóxicas, obtuvieron los CV más bajos, de 25.6% y 25.4% respectivamente.

Todos los factores que podían introducir variabilidad en los resultados fueron estudiados antes de la preparación de la serie de muestras enviadas en la Fase II. Para ésta se envió de nuevo un conjunto de muestras a cada uno de los laboratorios participantes, con un protocolo más detallado e instrucciones para evitar los factores de confusión encontrados en la Fase I. Los resultados de la Fase II se incluyen en la tabla 4 como porcentaje de pluteus normales (corregidos por el porcentaje de larvas normales en el control) así como X, SD y CV para cada muestra. Los resultados se representan en la figura 1 conjuntamente con el criterio para clasificar las muestras como

changing the mobility of contaminants to the liquid phase. A related factor is sample aeration, which can also be a critical step. High toxic effects were registered for sample A that had low chemical contamination (Casado-Martínez *et al.* 2006), possibly related to hydrogen sulphide. This compound, which is naturally present in anoxic sediments and can increase during sample storage, is very toxic for sea urchin embryos, and this toxicity can be falsely attributed to the sample itself (Lapota *et al.* 2000). The samples that could be affected by this factor are those from the port of Cádiz, samples A and B, which reported very variable results. Mean CV for Phase I was 61%, with values ranging from 9.7% for the negative toxicity control to 115% for sample B. Samples D and E, clearly not toxic, had the lowest CV, 25.6% and 25.4%, respectively.

All factors that could be identified were studied before Phase II was organized. A new set of samples was sent to each participating laboratory, including a detailed protocol and special guidelines to avoid interfering factors found during Phase I. The results are presented in table 4 as the percentage of normal pluteus corrected by the corresponding control and the X, SD and CV calculated for each sample. Figure 1 shows the results together with the toxicity criterion (decrease in percentage of normal pluteus higher than 20%) established based on previous results (DelValls *et al.* 2003). Except for the results reported by laboratory 3, which found higher toxicity for all the samples including the one with the lower chemical content, the laboratories reported more homogenous results than those for Phase I: all laboratories classified sample E' as highly toxic (for which high levels of organic compounds have been reported; Casado-Martínez *et al.* 2006), and samples A' (except for laboratory 3) and B' as not toxic. Samples C', D' and F' were classified as toxic by all laboratories, except for sample F' by laboratory 2, even though the percentages of normal pluteus varied greatly; thus, the selection of other toxicity criteria could introduce differences in sample classification. This variability in the percentage of normal pluteus was also found for the CV, which ranged from 12% to

Tabla 4. Porcentaje medio de éxito en la embriogénesis obtenido por los distintos laboratorios en los lixiviados de las muestras evaluadas durante la segunda fase del ensayo. X es la gran media, SD la desviación estándar y CV el coeficiente de variación en porcentaje.
Table 4. Mean percentage of normal pluteus for each laboratory and each sample of Phase II; X = grand mean, SD = standard deviation and CV = coefficient of variation.

Laboratory	Sample A'	Sample B'	Sample C'	Sample D'	Sample E'	Sample F'	Control
1	82	87	60	36	1	7	98
2	91	96	78	79	24	92	96
3	53	71	9	11	0	47	98
4	93	91	19	70	0	70	97
X	79.6	86.3	41.5	49.0	6.25	54.0	97.3
SD	18.5	10.8	32.9	31.4	11.8	36.3	0.96
CV	23.2	12.5	79.2	64.0	189	67.3	0.98
X[a]	88.7	91.3	52.3	61.7	8.3	56.3	97.0
SD[a]	5.9	4.5	30.2	22.7	13.6	44.1	1.0
CV[a] %	6.6	4.9	57.8	36.8	162.9	78.3	1.0

[a] Calculated excluding data from laboratory 3

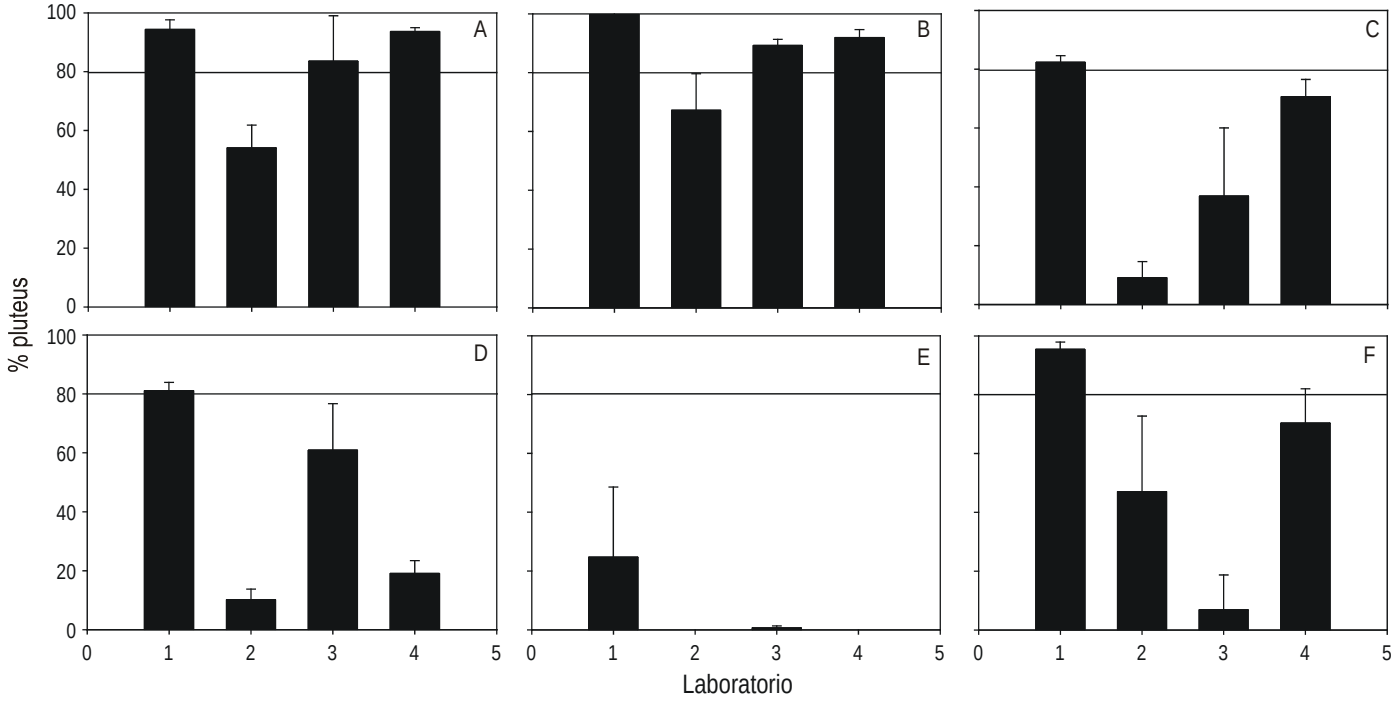

Figura 1. Resultados en porcentaje medio de pluteus normalmente desarrolladas para cada laboratorio y cada una de las muestras (nombradas de A a F). El 80% marca el umbral de toxicidad.
Figure 1. Mean percentage of normal pluteus for each laboratory and each of the samples (named A to F); 80% indicates the toxicity threshold.

tóxicas o no tóxicas (reducción del porcentaje de larvas pluteus normales superior a 20% respecto al control) establecido en base a resultados previos (DelValls *et al.* 2003). Excepto los resultados del laboratorio 3, en donde se encontraron niveles de toxicidad superiores en todas las muestras incluida la de menor contaminación, los de los otros tres laboratorios son más homogéneos que los obtenidos en la Fase I: todos los laboratorios consideraron la muestra E' (con niveles altos de compuestos de tipo orgánico; Casado-Martínez *et al.* 2006) 190% in this second phase. In Phase II, the mean percentage of normal pluteus in the controls was 97% and all laboratories met the acceptance criterion, with a CV value lower than 1%. The CV for samples A' and B' was 23% and 12%, respectively, according to the lower toxicity. For samples with intermediate toxicity, CV ranged from 64% to 79% and, as mentioned before, sample E' had the highest CV despite the homogeneous classification. The results of the statistical parameters calculated, excluding laboratory 3 that tended to report higher

altamente tóxica, y las muestras A' (excepto para el laboratorio 3) y B' no tóxicas. El resto de las muestras (C', D' y F') fueron consideradas tóxicas en todos los casos excepto la muestra F' en el laboratorio 2, aunque los porcentajes de larvas normales observados varían mucho entre laboratorios y por lo tanto una variación en el criterio de toxicidad podría hacer variar considerablemente la homogeneidad en la clasificación de las muestras de los laboratorios. Esta variabilidad en los porcentajes de larvas normales observados se refleja en los valores de CV, que varían entre 12% y 190% para esta segunda fase.

En el caso de la Fase II el porcentaje medio de larvas pluteus normales en los controles fue de 97% y en todos los laboratorios se superó el criterio de aceptabilidad del ensayo, con un CV menor a 1%. Para las muestras A' y B' el CV fue de 23% y 12% de acuerdo con la baja toxicidad registrada, las muestras con un grado de toxicidad intermedio obtuvieron CV entre 64% y 79% y, como se ha mencionado anteriormente, la muestra E' obtuvo la mayor variabilidad aunque los resultados sean más semejantes en la clasificación. Los resultados de los parámetros estadísticos calculados excluyendo los resultados del laboratorio 3 que parece evidenciar cierta tendencia anómala a registrar toxicidades superiores que el resto de laboratorios, reflejan una menor variabilidad especialmente para las muestras A', B' y C'.

Discusión

En general, se encontraron pocos laboratorios con infraestructura y un mínimo de experiencia en el desarrollo del ensayo con embriones de erizo de mar para la evaluación de la calidad de lixiviados de sedimentos. La variabilidad interlaboratorio de este ensayo, en estudios precedentes, ha sido de 63% cuando se calibra la EC50 (concentración efectiva que causa una disminución de la respuesta en el 50% de la población), valor similar a los registrados por los análisis químicos (Environment Canada 1992). Los resultados de este estudio son similares y, como en otros estudios, la variabilidad es mucho mayor cuando se ensayan sedimentos contaminados. En Holanda se han incluido dos ensayos para la evaluación de la toxicidad de lixiviados de sedimentos en los estudios de precisión interlaboratorio: el ensayo con embriones de ostra y el ensayo con rotíferos. Mientras el primero de ellos obtuvo CVs entre 34% y 210%, el ensayo con rotíferos fue considerado directamente no aconsejable ya que los resultados demostraron que era fundamental la experiencia previa para el desarrollo del ensayo (Stronkhorst 2003).

En nuestro estudio, el criterio para considerar las muestras como tóxicas o no tóxicas es doble e implica una diferencia estadística significativa ($P \leq 0.05$) y una reducción del porcentaje de larvas pluteus normales superior a 20% respecto al control negativo de toxicidad. El resultado de estos análisis estadísticos se incluye en la figura 2. La clasificación es homogénea entre laboratorios aunque se encuentran algunas diferencias entre las estaciones de toxicidad intermedia (F', C' toxicities than the other laboratories, show less variability especially for samples A', B' and C'.

Discussion

Few laboratories were found with the appropriate technology and experience to develop the bioassay using sea urchin embryos for sediment elutriate toxicity assessment. Previous studies reported interlaboratory variability of 63% when the calibrated endpoint was the EC50 value (effective concentration causing a response decrease of 50% in the total population), which is similar to the variability registered for chemical analyses (Environment Canada 1992). Our results are similar and, as in previous studies, variability is much higher when testing contaminated sediments. Two sediment elutriate toxicity bioassays were calibrated in the Netherlands, one using oyster embryos and the other using rotifers. The first showed a CV between 34% and 210%, while the second was considered not recommendable for regulatory purposes since the results showed that previous experience was essential for the successful development of the test (Stronkhorst 2003).

In this study, the criterion to consider samples toxic or not toxic is double and implies a statistically significant difference ($P \leq 0.05$) and a decrease of at least 20% of normal pluteus compared with the negative toxicity control. The results of the statistical analysis are summarized in figure 2. Sample classification is homogenous among laboratories although some differences were found for the intermediate toxic samples (F', C' and D'). Unrandom tendencies were observed for some laboratories and these tendencies appear to be related to the criteria established to consider larvae normal or abnormally developed; for example, laboratory 2 reported lower toxicity than the other laboratories and laboratory 3 found higher toxic responses in all samples. It seems that there is no confounding factor in the controls and in clearly toxic samples where embryos did not develop further than the early stages (gastrula), but this factor seems critical when toxicity levels arrest

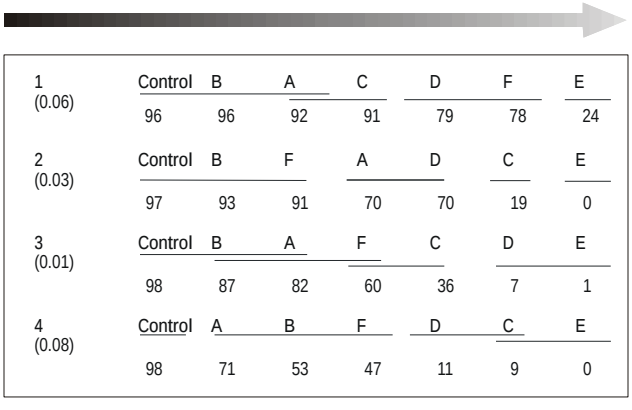

Figura 2. Resultado del análisis estadístico de los resultados del ejercicio de intercalibración del bioensayo de erizos de mar (estadístico de Levene entre paréntesis).

Figure 2. Results of the statistical analysis of the intercalibration exercise for the bioassay using sea urchin larvae (Levene's statistic in parentheses).

y D'). Se observaron determinadas tendencias en los resultados de algunos laboratorios y estas tendencias parecen ser debidas a los criterios utilizados por el operador para clasificar las larvas como normales o no normales. Por ejemplo, el laboratorio 2 tiende a detectar menor toxicidad que el resto de laboratorios mientras que el laboratorio 3 detecta siempre valores superiores. Parece no haber confusión en los controles o en las muestras claramente contaminadas donde los embriones no pasan de los primeros estadios de desarrollo (gástrula), pero este factor puede ser decisivo para niveles de toxicidad que interrumpen el desarrollo larvario en estados intermedios de desarrollo ya que el cambio exacto de pre-pluteus a pluteus es muy subjetivo (ver fig. 3). Por esta razón se recomienda la formación específica de los operadores.

Las diferencias entre los niveles de toxicidad interlaboratorio, pueden también deberse al contenido en material en suspensión de los lixiviados ya que éstos no fueron filtrados. La filtración no fue recomendada ya que puede introducir cambios o pérdidas de ciertos contaminantes (ASTM 1994). De cualquier modo, se ha demostrado que la presencia de material particulado puede afectar al desarrollo embrionario del erizo de mar y por tanto el resultado final (Carr 1998). Por ello, la centrifugación a altas velocidades para retirar el material particulado parece ser más recomendable en el caso de los ensayos realizados con el agua intersticial de los sedimentos (Ho *et al.* 1997). Del mismo modo, la centrifugación debe ser recomendable para evitar este factor de confusión y homogeneizar, en lo posible, los resultados entre laboratorios.

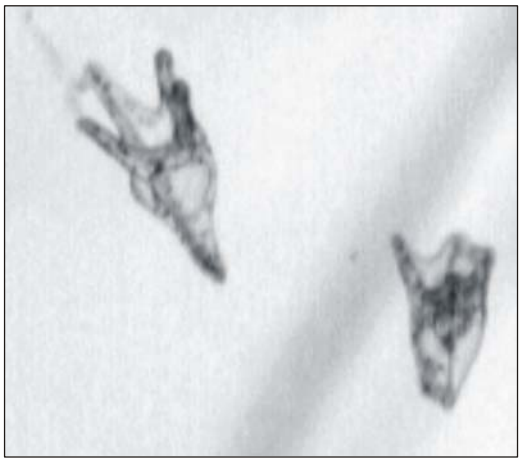

Figura 3. Ejemplo de estados de desarrollo donde el criterio del operador es crítico. A la izquierda una larva normal. A la derecha un embrión con las espículas y el aparato digestivo formado, pero donde los 4 brazos no están claramente separados como indica la definición de larva normal para este ejercicio.

Figure 3. Example of developmental stages where the selection criterion used to consider normal larvae is subjective: on the left, a normal larva; on the right, a developed embryo but with the four arms not completely separated.

Agradecimientos

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embryogenesis in the intermediate developmental stages since the change from prepluteus to pluteus is highly subjective (see fig. 3). It is therefore recommendable that the operators running this bioassay receive specific training if it is to be used for regulatory purposes.

The interlaboratory variability may also be a consequence of differences in suspended material in the elutriates since they were not filtered after settling. Filtration was not recommended because it can lead to loss of some contaminants (ASTM 1994). Nevertheless, it has been reported that particulate material can affect sea urchin embryo development and hence test results (Carr 1998), so centrifugation has been recommended when testing pore water (Ho *et al.* 1997). Centrifugation could therefore be suitable for testing sediment elutriates and to avoid this interfering factor.

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This study was carried out under a joint research project between CEDEX and the University of Cádiz. The Spanish Ministry of Science and Technology (REN2002_01699/TECNO) supported part of the work. The first author was supported by a grant (FPI) from the Spanish Ministry of Education and Science. We thank A Luque for their useful comments on the final manuscript.

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Nota de Investigación/Research Note

Ejercicio interlaboratorio de bioensayos marinos para la evaluación de la calidad ambiental de sedimentos costeros. IV. Ensayo de toxicidad sobre sedimento con crustáceos anfípodos

Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. IV. Whole sediment toxicity test using crustacean amphipods

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Resumen

Se estudió la precisión interlaboratorio del bioensayo con crustáceos anfípodos. Se recogieron nueve series de datos procedentes de distintos laboratorios europeos y de cuatro especies distintas (*Ampelisca brevicornis*, *Corophium volutator*, *Corophium multisetosum* y *Microdeutopus gryllotalpa)*, todas ellas usadas previamente para la caracterización de la toxicidad de sedimentos en España. Esos resultados se estudiaron para evaluar la variabilidad interlaboratorio de acuerdo a las diferentes especies y los distintos tiempos de almacenamiento de las muestras antes del ensayo. Los resultados mostraron coeficientes de variación similares a los encontrados en estudios previos y permitieron una clasificación homogénea de las muestras entre los laboratorios que completaron el ejercicio con éxito. No se identificaron tendencias significativas debidas al uso de distintas especies y, al parecer, el factor que puede afectar más críticamente la clasificación de las muestras y, por lo tanto, el uso de los resultados para la toma de decisiones, es el tiempo de almacenamiento de los sedimentos previamente al desarrollo del ensayo.

Palabras clave: material de dragado, bioensayo, calidad de sedimentos, España.

Abstract

Interlaboratory variability was studied for the bioassay using crustacean amphipods. Nine series of data were obtained from different laboratories using four different species (*Ampelisca brevicornis*, *Corophium volutator*, *Corophium multisetosum* and *Microdeutopus gryllotalpa)*, all previously used for sediment toxicity assessment in Spain. Results were studied for interlaboratory variability and according to different confounding factors. The coefficients of variation were similar to those previously reported for this bioassay and sample classification was homogeneous among the laboratories that succesfully completed the exercise. No significant tendencies related to test species were identified and it seems that the factor most critically affecting test results and the classification of samples is storage time before testing.

Key words: dredged material, bioassay, sediment quality, Spain.

Introducción

El bioensayo con crustáceos anfípodos se ha convertido en un ensayo de referencia para la caracterización de sedimentos contaminados y material de dragado, y es usado rutinariamente para evaluar los efectos biológicos potenciales de este tipo de muestras ambientales. Existen en la actualidad protocolos estandarizados (ASTM 1991, Environment Canada 1992, USEPA 1994, RIKZ 1999) para distintas especies (p.e.

Introduction

The bioassay using crustacean amphipods has become a benchmark for contaminated marine sediments and dredged material characterization. It is routinely used to measure the biological effects of sediment samples and standard operating procedures already exist (ASTM 1991, Environment Canada 1992, USEPA 1994, RIKZ 1999) for some species (e.g., *Corophium volutator, Ampelisca abdita, Rhepoxynius*

Corophium volutator, Ampelisca abdita, Rhepoxynius abronius) pero se han utilizado otras especies autóctonas con algunas modificaciones a las condiciones de ensayo (Costa *et al.* 1998, Onorati *et al.* 1999). En España se han usado con éxito distintas especies para la caracterización de sedimentos y materiales de dragado: *Corophium volutator* (Casado-Martínez *et al.* 2004), *C. multisetosum* (Belzunce *et al.* 2004), *Ampelisca brevicornis* (Riba *et al.* 2003, 2004) y *Microdeutopus gryllotalpa* (DelValls *et al.* 1998, César *et al.* 2002). La principal razón de la variabilidad en la selección de la especie con la que se realiza el ensayo radica en la distribución a lo largo de las costas españolas de cada una de ellas, ya que su abundancia depende de la zona geográfica y de su disponibilidad a lo largo del año. Aunque todas ellas parecen recomendables para la caracterización de materiales de dragado y el efecto de la sensibilidad en la toma de decisiones puede ser evitado mediante el uso de análisis estadísticos para la clasificación de las muestras como tóxicas o no tóxicas, otros factores pueden ser causantes de toxicidad y deben tenerse en cuenta para el análisis de los resultados. Entre éstos, el tamaño de grano parece tener efectos en algunas especies de anfípodos. Por ejemplo *Rhepoxynius abronius* parece verse afectado por sedimentos muy finos y aunque estas diferencias pueden ser mínimas cuando se estudian sedimentos contaminados, la supervivencia puede llegar a reducirse en un 15% cuando los porcentajes de limos y arcillas son del 80% aun en ausencia de contaminación (Tay *et al.* 1998). Cabe mencionar que estos porcentajes de sedimentos de tamaños finos son comunes en muestras de materiales de dragado (DelValls *et al.* 2001, 2003).

Para el ejercicio se distribuyó un protocolo general a cada uno de los laboratorios participantes ya que la mayoría de ellos disponía de personal experimentado en el uso de este ensayo de toxicidad. Los principales factores que pueden causar variabilidad en los resultados interlaboratorio son el uso de distintas especies de anfípodos de distintas poblaciones que, por lo tanto, estuvieron sujetas a distinta aclimatación, manipulación y mantenimiento de los organismos. Los objetivos principales fueron evaluar la habilidad de los distintos laboratorios para caracterizar sedimentos contaminados como tóxicos o no tóxicos, determinar diferencias en la supervivencia media registrada en los distintos laboratorios y detectar las causas de estas diferencias. En caso de introducir cualquier modificación al protocolo común, ésta se incluyó en la memoria de los resultados. Otros factores, como el tiempo de almacenamiento, han sido estudiados para intentar esclarecer su posible influencia en la toxicidad registrada.

Material y métodos

Los anfípodos fueron recogidos por los laboratorios participantes en las zonas donde cada uno los recogía normalmente para sus ensayos de toxicidad. En todos los casos se trataba de áreas libres de contaminación. Los organismos se aclimataron a las condiciones del laboratorio en acuarios de 20 L con unos 3 cm de sedimento aproximadamente y agua de mar, y con

abronius), though other amphipod species can be used with minor modifications (Costa *et al.* 1998, Onorati *et al.* 1999). In Spain, the following species have been successfully used for sediment and dredged material toxicity assessment: *Corophium volutator* (Casado-Martínez *et al.* 2004), *C. multisetosum* (Belzunce *et al.* 2004), *Ampelisca brevicornis* (Riba *et al.* 2003, 2004) and *Microdeutopus gryllotalpa* (DelValls *et al.* 1998, César *et al.* 2002). The main reason for this variability in test species selection is their distribution along the coasts of Spain, the presence or absence of certain species depending on the geographical zone and their year-round availability. Even though all of them are recommended for dredged material characterization and the effect of different sensitivity for making pass/fail decisions in regulatory programs can be avoided by using statistical analyses for sample toxicity classification, other factors, such as particle size distribution, may cause toxic effects. For example, *Rhepoxynius abronius* can be affected by very fine sediments and even if the effect on toxicity is lower when testing contaminated sediments, survival can decrease by 15% when the silt-clay content is greater than 80% (Tay *et al.* 1998), which is common for dredged sediment samples (DelValls *et al.* 2001, 2003).

For this study, a protocol based on standard procedures was sent together with the sediment samples to all the participating laboratories, most having staff trained in sediment toxicity assessment using amphipods. Variability factors included different species of amphipods and different populations, acclimation and handling. The objectives were to evaluate the ability of the different laboratories to characterize contaminated sediments according to toxicity, to determine differences in mean survival between laboratories and to detect the causes of such differences. Any change made to the general protocol was reported with the test results. Other factors such as time storage have been studied to try to identify their contribution to sediment toxicity.

Material and methods

Toxicity tests were applied to six sediment samples provided by the University of Cádiz in 2003 (Casado-Martínez *et al.* 2006). The amphipods used in the bioassays were obtained by each laboratory from where they normally collect them for routine toxicity analysis, in all cases from clean areas free of contamination. Organisms were acclimated to laboratory conditions in 20-L aquaria with a 3-cm sediment bottom layer and clean seawater, and aeration was adjusted to ensure enough dissolved oxygen without disturbing the sediment. They were fed a mixture of microalgae or commercial food (depending on the laboratory) once a week and water was changed on the following day. During acclimation, the dissolved oxygen concentration, pH, salinity and temperature were controlled.

The bioassay using amphipods was a 10-day static test on whole sediment. Test parameters and conditions are given in table 1, though some slight modifications were introduced depending on the test species. Sediments were added to the test

Tabla 1. Parámetros y condiciones a seguir para el desarrollo del test con crustáceos anfípodos en el laboratorio.
Table 1. Parameters and conditions to develop the test using crustacean amphipods in the laboratory.

Parameters	Conditions
1. Test type	Static; on whole sediment
2. Temperature	15–20ºC (depending on the species)
3. Salinity	32–40
6. Photoperiod	Natural of the season; also continuous light
7. Test chambers	Glass, 2 L (recommended cylindrical and covered)
8. Volume of sediment	250 mL (or 1:4 sediment/water)
9. Volume of overlying water	1 L (or 1:4 sediment/water)
10. Water renewal	No
11. Size and state of organisms	*Ampelisca brevicornis*, 3–5 mm; *Corophium volutator*, 5 mm or larger; *Mycrodeutopus gryllotalpa*, 5 mm or larger; *Corophium multisetosum*, 10 mm
12. Number of organisms per chamber	20
13. Number of replicates	3–5
14. Feeding regime	No
15. Aeration	12 h before introducing the organisms, to ensure dissolved oxygen concentrations equal or higher than 90% saturation
16. Overlying water	Clean seawater
17. Water quality	pH, ammonia, salinity and dissolved oxygen at the beginning and at the end of the test
18. Test duration	10 days
19. Endpoints	Survival
20. Test acceptability	90% survival in the negative toxicity control

aireación ajustada para no remover el sedimento del fondo. Los organismos se alimentaron con una mezcla de microalgas o alimento comercial (dependiendo del laboratorio) una vez por semana y el agua sobrenadante se cambió al día siguiente. Durante el periodo de aclimatación se controlaron el pH, la concentración de oxígeno disuelto, la salinidad y la temperatura.

El ensayo con anfípodos se desarrolló a 10 días de exposición en condiciones estáticas y sobre el sedimento en bruto. Los parámetros para la realización del ensayo en el laboratorio se incluyen en la tabla 1, aunque se tuvieron en cuenta ciertas modificaciones en las condiciones dependiendo de la especie utilizada. Los sedimentos se añadieron a las cámaras de exposición hasta una profundidad de 3 cm y posteriormente se les añadió agua de mar en relación 1:4 v/v sedimento:agua. Una vez que los sedimentos hubieron decantado se inició la aireación al menos durante 12 h previamente a la introducción de los organismos. Se usaron 20 anfípodos por replica y un mínimo de tres replicas por muestra. Pasados los 10 días de exposición se registró el porcentaje de supervivencia mediante el tamizado (0.5 mm) de las muestras y se calculó el porcentaje medio de mortalidad en cada una. Los organismos desaparecidos se consideraron como muertos.

Para evaluar la variación interlaboratorio se ha calculado el coeficiente de variación (CV) para cada muestra como el

vessels until attaining a 3-cm bottom layer and clean seawater was added at a ratio of 1:4 v/v (sediment:water). They were left to settle and then aerated for at least 12 h before amphipods were added. Twenty randomly-selected amphipods were used per replicate and each sample consisted of a minimum of three replicates. After the 10-day exposure period, the percentage of surviving organisms was estimated by sieving the samples through a 0.5-mm mesh. Mean mortality was calculated for each sample. Missing organisms were considered dead.

To determine the interlaboratory precision, the coefficient of variation (CV) was calculated for each sample by dividing the standard deviation (SD) by the mean for all laboratories (X):

$$CV\ (\%) = \frac{SD}{X} \cdot 100$$

The effect of interlaboratory variability on the sample classification was also evaluated according to the individual test results for each laboratory. Percentage of mortality was normalized by angular transformation and treated as a normal distribution. Statistically significant differences ($P \leq 0.05$) between a negative toxicity control (sample A) and the sediment samples were established by means of an ANOVA and Tukey's test using the SPSS 11.0 program.

cociente entre la desviación estándar (SD) y la media de los laboratorios (X):

$$CV\ (\%) = \frac{SD}{X} \cdot 100$$

Además se estudió el efecto de esta variabilidad en la clasificación de las muestras según los resultados del ensayo en cada laboratorio individualmente. El porcentaje de mortalidad se normalizó mediante transformación angular y fue tratado como una distribución normal para posteriormente buscar diferencias entre el control negativo de toxicidad (sedimento A) y cada una de las muestras. Las muestras significativamente homogéneas ($P \leq 0.05$) fueron identificadas mediante un test ANOVA y test de Tukey mediante el programa estadístico SPSS 11.0.

Resultados

En total se recogieron datos de nueve laboratorios participantes. Los laboratorios (identificados del 1 al 9), las fechas de ensayo y las especies utilizadas por cada uno de ellos se presentan en la tabla 2. Se encontraron diferencias importantes en el tiempo de almacenaje de las muestras de sedimento previamente al inicio del ensayo, muy por encima de los tiempos recomendados que son de tres a cuatro semanas (USEPA 1994), sin embargo, por diversas razones este tiempo varió desde las tres semanas hasta tres meses para algunos laboratorios. Los resultados de mortalidad media y la desviación estándar se presentan en la figura 1, en la que se aprecia la disminución de la toxicidad registrada por los laboratorios 7, 8 y 9 para todas las muestras excepto la F. Todos los laboratorios excepto el 6 y el 9 alcanzaron los valores mínimos de supervivencia en el sedimento control para la aceptabilidad del ensayo (90%). Por ello los resultados de los laboratorios 6 y 9 no fueron considerados para el cálculo de los parámetros estadísticos. Los parámetros de calidad del agua (concentración de oxígeno disuelto, pH, salinidad, temperatura y amonio total) estuvieron en el rango permitido para las especies.

Los resultados del análisis para identificar las muestras estadísticamente homogéneas ($P \leq 0.05$) se muestran en la figura 2. Como puede observarse, todos los laboratorios clasifican a la muestra A como no tóxica y a las muestras F y C como las más tóxicas. El resto de las muestras (B, D y E) podrían ser clasificadas como moderadamente tóxicas aunque se observan diferencias en sus valores de mortalidad y en su clasificación respecto al resto de muestras.

Los resultados del estudio interlaboratorio se resumen en la tabla 3. Si incluimos para el cálculo todos los resultados excepto aquellos que no cumplen los criterios de aceptabilidad para el control negativo, el coeficiente de variación interlaboratorio fue alto para la muestra A (114.5%) debido principalmente a los bajos valores de mortalidad en todos los laboratorios, que distorsionan el valor de la estimación de la

Results

Data sets were collected from nine participating laboratories (numbered from 1 to 9); the test dates and test species are shown in table 2. Important differences were reported for the sample storage period before toxicity testing, some laboratories failing available guidelines (USEPA 1994). Recommended storage time is three to four weeks, but for diverse reasons it ranged from three weeks to three months. Mean mortality results and standard deviation are represented in figure 1. A decrease in sediment toxicity can be observed for laboratories 7, 8 and 9 for all the samples except F. All the laboratories except 6 and 9 registered mortality values for the negative toxicity control under the maximum guideline for test acceptability (10%); hence, the results of these two laboratories were not considered for further analyses. Water quality parameters (dissolved oxygen concentration, pH, salinity, temperature and total ammonia) were in the range where no adverse effects are expected on test organisms.

The results of the statistical analysis to identify homogeneous responses ($P \leq 0.05$) are shown in figure 2. All laboratories classified sample A as not toxic and samples F and C as the most toxic. Samples B, D and E could be considered moderately toxic, though some differences were observed regarding the percentage of mortality and the classification relative to the rest of the samples.

The results of the interlaboratory precision study are summarized in table 3. When the statistics were calculated including all the results except those not meeting the acceptance criteria, a high CV was obtained for sample A (114.5%), mainly because of the low mean mortality that increased this value. The rest of the samples had CVs ranging from 19.8% (sample F) to 69.8% (sample D). When the test results from laboratories that reported storage periods longer than four weeks were also excluded from the calculations, the CVs ranged from 10.5% (sample F) to 81.8% (sample A), with a

Tabla 2. Días transcurridos desde la recogida de los sedimentos y el inicio del ensayo, y especie utilizada en cada laboratorio.
Table 2. Days after sampling when the test was started and species used at each participating laboratory.

Laboratory	Days after sampling	Species
1	1 day	*Ampelisca brevicornis*
2	1 day	*Corophium* sp.
3	2 days	*Corophium multisetosum*
4	8 days	*Ampelisca brevicornis*
5	15 days	*Mycrodeutopus gryllotalpa*
6	60 days	*Corophium* sp.
7	65 days	*Corophium multisetosum*
8	90 days	*Corophium volutator*
9	110 days	*Corophium* sp.

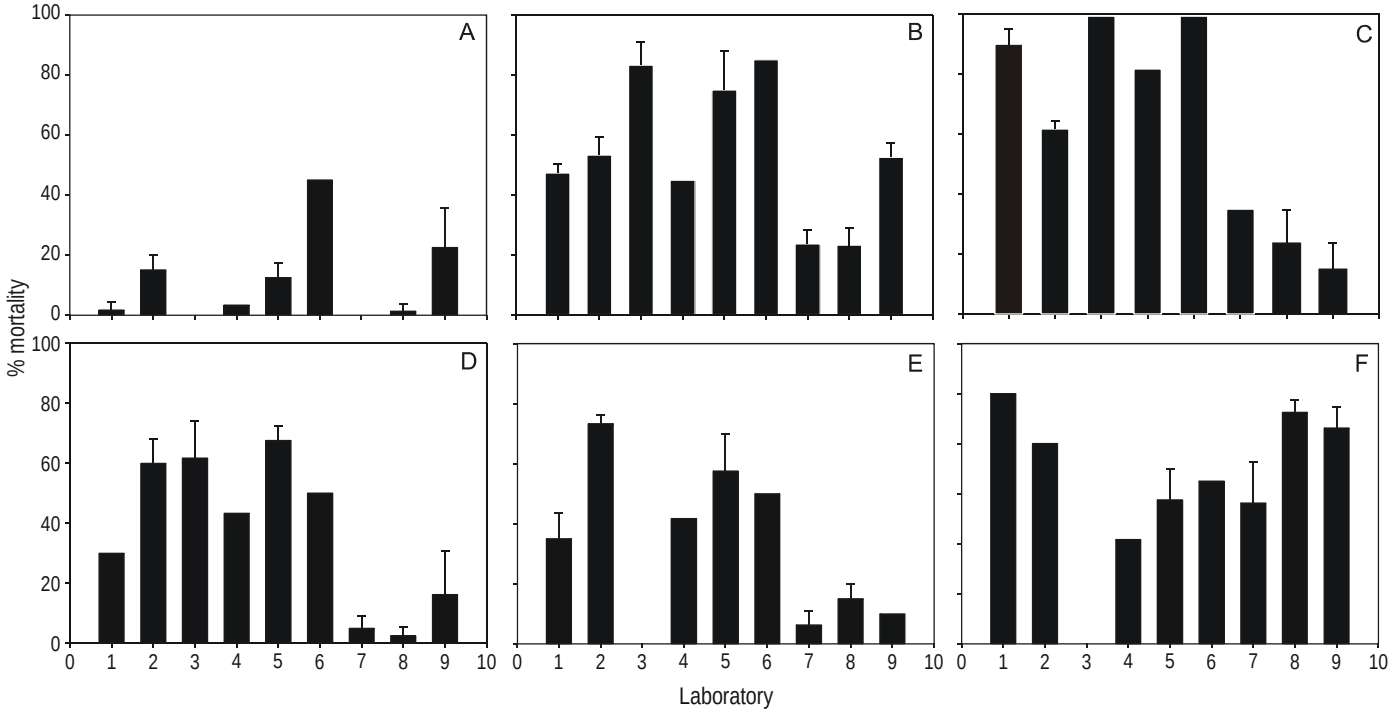

Figura 1. Resultados en porcentaje medio de mortalidad y desviación estándar para cada laboratorio y cada una de las muestras. Los laboratorios están numerados de 1 a 9 y las muestras de A a F.

Figure 1. Mean percentage of mortality and standard deviation for each laboratory and each sample. Laboratories are numbered from 1 to 9 and samples from A to F.

variabilidad mediante este método. Con el resto de las muestras el CV varía entre 69% y 19%, para las muestras D y F respectivamente. Si se calculan estos parámetros excluyendo también los laboratorios que desarrollaron el ensayo fuera del tiempo máximo de almacenamiento recomendado (cuatro semanas) los CV se encuentran entre 81% para la muestra A y 10.5% para la muestra F, con un CV medio del 33% (23% si excluimos la muestra A). Con todas las muestras se obtuvieron valores medios de mortalidad mayores a 50% excepto con la muestra A.

Discusión

El bioensayo con crustáceos anfípodos se realizó con éxito en casi todos los laboratorios. Excepto dos laboratorios (los laboratorios 6 y 9) que superaron los valores de mortalidad permitidos para el control de toxicidad negativo, el resto de los resultados se consideraron aceptables y no se encontraron factores importantes de confusión para el análisis de dichos resultados. Si el laboratorio tiene cierto nivel de experiencia previa, como en la mayoría de los casos, el ensayo puede ser desarrollado con facilidad. Cabe mencionar que en uno de los laboratorios cuyos resultados quedaron invalidados por la elevada mortalidad en el control negativo de toxicidad era la primera vez que se realizaba el ensayo.

El ensayo parece adecuado para la caracterización en laboratorio de la toxicidad de muestras de dragado ya que los

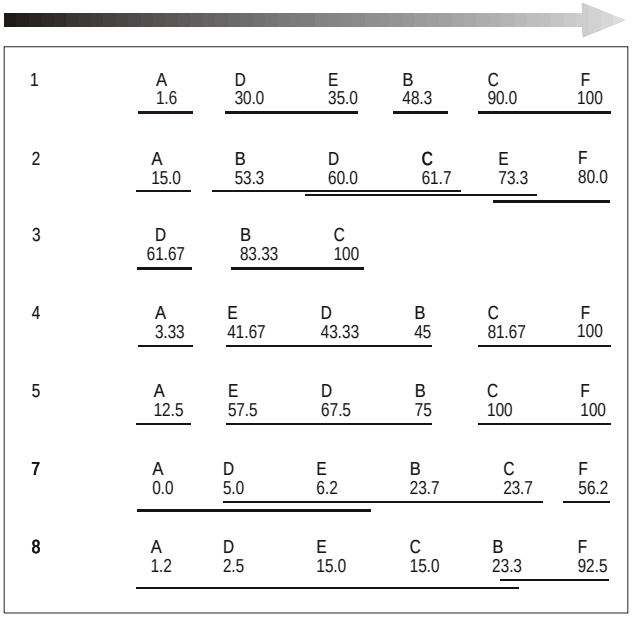

Figura 2. Resultado del análisis estadístico de los porcentajes de mortalidad de cada laboratorio (excepto aquellos cuya supervivencia en el control no cumplía los criterios de aceptabilidad) del ejercicio de intercalibración del bioensayo con anfípodos ($P \leq 0.05$).

Figure 2. Results of the statistical analysis of the mortality percentages for each laboratory (except those whose control survival did not meet the acceptance criteria) during the intercalibration exercise of the bioassay using amphipods ($P \leq 0.05$).

Tabla 3. Variabilidad interlaboratorio de los resultados de mortalidad media del ensayo de toxicidad con crust·ceos anfÌpodos. n.a. = dato no disponible.
Table 3. Interlaboratory variability of mean mortality from sediment toxicity tests with crustacean amphipods; n.a. = not available.

Laboratory	Sample					
	A	B	C	D	E	F
1	1.6	47.3	90.0	30.0	35.0	100.0
2	15.0	53.3	61.7	60.0	73.3	80.0
3	n.a.	83.3	100.0	61.7	n.a.	n.a.
4	3.33	45.0	81.7	43.3	41.7	100.0
5	12.5	75.0	100.0	67.5	57.5	100.0
6	45.0	85.0	35.0	50.0	50.0	65.0
7	0.0	23.8	23.8	5.0	6.25	56.3
8	1.3	23.3	15.0	2.5	15.0	92.5
9	22.5	52.5	n.a.	16.3	10.0	86.3
SD[a]	6.43	23.0	35.4	26.9	25.3	17.5
Xm[a]	5.62	50.2	67.4	38.6	38.1	88.1
CV[a]	114.5	45.9	52.5	69.8	66.2	19.8
SD[b]	6.63	17.3	15.9	15.5	17.1	10.0
Xm[b]	8.11	60.8	86.7	52.5	51.9	95.0
CV[b]	81.8	28.4	18.4	29.4	33.0	10.5

[a] SD, Xm and CV include all data points except those for laboratories 6 and 9.
[b] SD, Xm and CV include only sediments meeting the control survival criterion and not tested at a later date.

laboratorios clasificaron eficazmente las muestras de acuerdo con la contaminación presente (Casado-Martínez *et al.* 2006). Dentro de la reglamentación para el proceso de este tipo de sedimentos se han usado distintas aproximaciones para la clasificación de las muestras según la respuesta del ensayo de toxicidad. Algunos países han optado por criterios absolutos en forma de valores de mortalidad que clasifican las muestras como tóxicas si éstos son superados (por ejemplo el Reino Unido considera tóxicas las muestras con mortalidades superiores al 40% en el ensayo con *Corophium volutator*). Otras agencias utilizan el doble criterio de un valor mínimo de mortalidad superior a un sedimento de referencia o control y además la diferencia estadística (den Besten 2003). En este caso se ha utilizado el criterio del 20% de mortalidad superior al sedimento control y la diferencia estadística para considerar una muestra como tóxica (USEPA 1998, Environment Canada 2000). Como se ha mencionado anteriormente, la clasificación de las muestras según su toxicidad fue similar en todos los laboratorios aunque ciertamente existe variabilidad en los porcentajes de mortalidad registrados en cada uno de ellos. Una de las causas de esta variabilidad es el tiempo de almacenamiento de las muestras que parece provocar la disminución de la toxicidad medida. El laboratorio 7, que realizó el ensayo cuatro semanas después del muestreo de los sedimentos, obtuvo resultados de toxicidad para las muestras D y E estadísticamente similares ($P \leq 0.05$) al control de toxicidad negativo y las muestras B y C podrían ser consideradas moderadamente tóxicas. Cuando el tiempo de almacenamiento aumenta a más de cuatro semanas, como es el caso del laboratorio 8, sólo los mean CV of 33% (23% excluding A, the negative toxicity control). Mean mortalities greater than 50% were obtained for all the samples except for A.

Discussion

The bioassay using crustacean amphipods was carried out successfully by most of the laboratories. Except for two laboratories (6 and 9) that failed the acceptance criteria due to higher mortalities in the negative toxicity control, the test results were acceptable since no interfering factors were found. One of the laboratories that did not meet the acceptance criteria was performing the test for the first time.

These results indicate that the test is suitable for dredged material characterization since laboratories classify the samples based on sediment contamination. Different approaches have been used for regulatory processes to classify sediment samples according to the toxic response. Some countries are using an absolute mortality value to classify the samples as toxic. In the UK, for example, samples are considered toxic when the mortality values are higher than 40% in the test using *C. volutator.* Other agencies use two criteria: a minimum percentage of mortality higher than the control or reference sediment and the statistically significant difference (den Besten 2003). In this exercise samples were classified as toxic based on 20% mortality higher than the control sediment and the statistical difference (USEPA 1998, Environment Canada 2000). As mentioned before, the classification of samples according to the toxicity was similar for all laboratories despite some

efectos producidos por la muestra F serían considerados como estadísticamente diferentes al sedimento control ($P \leq 0.05$), mientras que las otras cuatro muestras C, E, B y D se considerarían, al igual que la muestra A ($P \leq 0.05$), no tóxicas. Dado que el aumento del tiempo de almacenamiento parece afectar la toxicidad registrada y, por lo tanto, la clasificación de los materiales, éste debería ser un factor crítico a considerar por las agencias reguladoras para aceptar los resultados. Aunque los resultados de este ejercicio deben tomarse con precaución, cabe destacar que los resultados de los ensayos realizados tras ocho semanas desde la recogida de las muestras ya no obtuvieron la misma clasificación de los sedimentos, clasificando como no tóxicos materiales de categoría III con altas concentraciones químicas (Casado-Martínez *et al.* 2006).

En cuanto a la variabilidad de los resultados, el valor de CV encontrado con la muestra A, considerada control de toxicidad negativo, es notablemente superior a los valores encontrados con el resto de las muestras. Esto no significa que esta variabilidad afecte al análisis de los resultados obtenidos ya que los resultados clasifican claramente en todos los casos la muestra como no tóxica, sino más bien refleja los inconvenientes del uso de este valor para estimar la variabilidad, ya que si expresásemos los resultados como porcentaje de supervivencia, el CV tomaría valores muy elevados con aquellas muestras con valores medios más bajos, que en este caso corresponderían a las muestras más tóxicas. Del mismo modo parece haber mayor variabilidad en el ensayo de muestras medianamente tóxicas, la cual disminuye al aumentar la toxicidad: las muestras D y E, con valores de mortalidad de ~50%, obtuvieron CV ~30%; con la muestra B la mortalidad fue de 60% y el CV de 28%; mientras que las muestras C y F, las más tóxicas de las analizadas con valores medios de mortalidad de 85% y 95%, registraron menor variabilidad con CVs de 18% y 10%, respectivamente.

La posibilidad de falsos negativos, entendidos como sedimentos tóxicos que son clasificados por un ensayo como no tóxicos, debe ser estudiada por sus implicaciones ambientales. En este sentido, parece que el mayor factor que puede hacer aumentar el número de falsos negativos es el aumento del tiempo de almacenamiento. Por el contrario los falsos positivos, entendidos como los sedimentos no tóxicos clasificados como tóxicos, son también de importancia cuando se deben usar los resultados de estos ensayos para la toma de decisiones, especialmente si han de ser utilizados como principal herramienta legislativa en procesos de gestión. Para minimizar el número de decisiones erróneas y, por lo tanto, los costes ambientales y económicos asociados a este tipo de actividades, deben conocerse los factores que pueden aumentar la toxicidad de las muestras; es decir, los factores que pueden aumentar el número de falsos positivos asociados al método. No sólo la variabilidad y la naturaleza de los sedimentos van a causar efectos tóxicos. Existen numerosos factores que pueden enmascarar la respuesta tóxica, como por ejemplo cambios en el pH o la presencia de determinados compuestos como sulfuros o amonio, aunque una correcta caracterización de la

variability in the mortality values. One of the reasons for this variability is the storage period, which appearently causes a decrease in sediment toxicity. Laboratory 7 tested the sediments after four weeks and classified samples D and E as statistically similar ($P \leq 0.05$) to the negative toxicity control, while samples B and C could be considered moderately toxic. If the storage time is longer than four weeks, as occurred at laboratory 8, only sample F is considered statistically different from the control ($P \leq 0.05$) and samples C, E, B and D would be classified as not toxic ($P \leq 0.05$). Since an increase in sample storage time seems to change the toxic response and its classification, it should be considered a determining factor to accept test results for regulatory purposes. Although these results must be taken with caution, we could say that the results obtained after a certain storage time may not classify the sediments similarly, and even sediment classified as category III due to high contaminant concentrations (Casado-Martínez *et al.* 2006) would be considered not toxic.

The results of the variability study produced the highest CV for sample A, the negative toxicity control. This variability did not affect the analysis of the results because all laboratories classified this sample as not toxic, but the inconvenience of using this statistic to estimate variability is evident. If the results were to be expressed as survival values, the CV would be extremely high for those samples reporting the lowest mean values that would be attributed to the most toxic samples. At the same time, it seems that moderately toxic samples are related to a higher variability, which decreases with an increase in toxicity: samples D and E, with mean mortality values of 50%, obtained CVs of ~30%; sample B had 60% mortality and a CV of 28%; and samples C and F, the most toxic samples with mortalities of 85% and 95%, respectively, had CVs of 18% and 10%, respectively.

The possible false nontoxic sediments (toxic sediments classified as not toxic according to test results) should be studied to avoid adverse environmental effects related to contaminated sediments and dredged material disposal. Storage time seems to be an important factor that can increase the number of these false negatives. On the other hand, false toxic sediments (not toxic sediments classified as toxic according to test results) are also important when using a bioassay for decision-making and especially if they are to be used as principal regulatory tool for managing dredged materials. If we want to minimize incorrect decisions and the related environmental and economic costs, we have to minimize the number of false positives related to the method and to study the factors that are actually increasing their number. It is not only the variability and nature of sediments that cause toxicity. Many other factors can also influence the toxic response, such as pH changes or certain compounds such as hydrogen sulphides or ammonia. A correct water quality characterization can be helpful for early identification and to ensure that concentrations are among the acceptance criteria. Another possible confounding factor is the grain size distribution, since the species used in this study live in sediments with different characteristics: *A. brevicornis* and

calidad del agua puede ayudar a identificar estos factores y asegurar que se encuentran dentro de los límites de tolerancia para la especie. Otro posible factor de confusión es la distribución de tamaños de grano del sedimento. Las distintas especies utilizadas se encuentran normalmente en sedimentos de distinta naturaleza: las especies *Ampelisca brevicornis* y *Corophium multisetosum* son características de sedimentos arenosos (<5% de limos <63 µm) pero *Corophium volutator* predomina en sedimentos limosos (contenido en limos <63 µm mayor al 20%). Se han realizado distintos estudios para identificar posibles efectos adversos de este factor y para establecer los rangos óptimos para determinadas especies. Un estudio realizado por la agencia ambiental canadiense incluso estableció una clasificación de las especies recomendadas en ese país según el grado de sensibilidad al tamaño de grano (Environment Canada 1992). Dado que los resultados de este ejercicio no pueden llevar a conclusiones de este tipo, se recomienda el estudio de los resultados en cada caso y de acuerdo a los factores que pueden afectar a la especie utilizada hasta que no se disponga de rangos de aplicabilidad y/o recomendaciones explícitas por parte de las agencias competentes.

En general, se puede considerar que los resultados de este primer ejercicio son similares a los obtenidos previamente en otros ejercicios interlaboratorio aún cuando se incluyen los resultados de ensayos realizados tras 8 y 12 semanas del muestreo de los sedimentos (CV = 61%). Cuando se cumplen todos los criterios de aceptabilidad, el valor medio de CV de 33% está incluso por debajo del valor de 46% desarrollado por Parkhurst *et al.* (1992) tras la revisión de la variabilidad en ensayos agudos realizados con compuestos químicos individuales. Este último valor, que se desarrolló con resultados para distintas especies, puede ser considerado como el CV esperado cuando se realizan ensayos de toxicidad debido al factor biológico (Still *et al.* 2000). Además, la variabilidad en nuestros resultados es similar a la obtenida con *C. volutator* por Bowmer (1993), quien registró CVs entre 39% y 52% aunque con sedimentos contaminados artificialmente y gran experiencia de los laboratorios en el desarrollo del ensayo. En el mismo rango, Schlekat *et al.* (1995) estudiaron la variabilidad para el ensayo con *Eohaustorius estuarius* (CV de 1.5–45%), *Leptocheirus plumosus* (CV de 5–65%) y *Ampelisca abdita* (CV de 5–146%) y Mearns *et al.* (1986) con *Rhepoxynius abronius* obtuvieron CVs de 6–81%. Aunque la posible influencia de la experiencia en el desarrollo del ensayo en los resultados y en su interpretación puede evitarse mediante cursos de perfeccionamiento, es más difícil encontrar especies comunes para las distintas zonas geográficas; no obstante, parece que el uso del doble criterio para la clasificación de las muestras como tóxicas puede evitar la variabilidad debida al uso de distintas especies con distintas sensibilidades.

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C. multisetosum are usually found in sandy sediments (<5% silt <63 µm), but *C. volutator* predominantly occurs in silty sediments (content of silt <63 µm higher than 20%). Different studies have focused on identifying the possible adverse effects due to this factor and to establish the guidelines for each species. The Canadian Environmental Agency published a classification of test species according to their preferences for grain size distribution (Environment Canada 1992). Since our results are not so conclusive, it would be recommendable to study the results case by case and together with the factors affecting the test species until applicability ranges can be established by the interested agencies.

The results of this first interlaboratory exercise in Spain are similar to previous test results, even including those reported after 8 and 12 weeks (CV = 61%). Mean CV was 33% for the laboratories that met the acceptance criteria; this is lower than the value of 46% reported by Parkhurst *et al.* (1992) after reviewing the variability of acute responses to particular chemicals. This value, developed using different species, can be considered the expected CV when using biological toxicity tests (Still *et al.* 2000). Similar results were reported by Bowmer (1993) using *C. volutator* (CVs of 39–52%), spiked sediments and highly trained laboratories. In the same range, Schlekat *et al.* (1995) used *Eohaustorius estuarius* (CVs of 1.5–45%), *Leptocheirus plumosus* (CVs of 5–65%) and *Ampelisca abdita* (CVs of 5–146%), and Mearns *et al.* (1986) used *Rhepoxynius abronius* (CVs of 6–81%). The variability associated with the different experience of the laboratories can be avoided through training courses and further experience, but it is more difficult to find common available species; thus, it seems that the double criterion method used for sediment toxicity classification is suitable to avoid differences in test species selection and different sensitivities.

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Nota de Investigación/Research Note

Ejercicio interlaboratorio de bioensayos para la evaluación de la calidad ambiental de sedimentos costeros. V. Ensayo de toxicidad sobre sedimento con juveniles del bivalvo *Ruditapes philippinarum*

Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. V. Whole sediment toxicity test using juveniles of the bivalve *Ruditapes philippinarum*

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Resumen

Este estudio resume los resultados del ejercicio interlaboratorio realizado en tres laboratorios para evaluar el uso del ensayo con la almeja comercial de Manila *Ruditapes philippinarum.* Seis muestras de sedimentos dragados se estudiaron mediante dos medidas finales distintas: mortalidad tras dos periodos de exposición distintos (7 y 14 días) y la medida subletal que estudia el porcentaje de organismos enterrados tras 48 h desde el inicio de la exposición. La medida de la letalidad fue sólo sensible tras el periodo más largo de exposición y la contaminación metálica más elevada. La actividad de enterramiento mostró resultados muy variables y altamente dependientes del operador responsable. De acuerdo con estos resultados se recomienda la revisión del protocolo para mejorar su uso en la gestión de dragados portuarios especialmente si se trata de zonas no afectadas por contaminación metálica.

Palabras clave: almeja de Manila, material de dragado, tasa de enterramiento.

Abstract

Several species of bivalves and procedures have been used to characterize sediment toxicity. Here we report the results of an interlaboratory exercise that included three different laboratories to evaluate the use of the bioassay using the commercial clam *Ruditapes philippinarum*. Six different dredged sediments were studied using two different endpoints: lethality after two different exposure periods (7 and 14 days) and burrowing activity after 48 h of exposure. The lethal endpoint was only sensitive to characterize samples with high metallic concentration and following the 14-day exposure period. The burrowing activity showed very variable results that evidence the unsuitability of this endpoint for dredged material characterization. According to these results, a new design is recommended for the test using juvenile bivalves if it is to be used to characterize sediment samples on a regulatory context especially if sediments are not affected by metallic contamination.

Key words: Manila clam, dredged material, burrowing activity.

Introducción

Varias especies de moluscos bivalvos han sido identificadas como indicadores de contaminantes, especialmente de origen metálico, en sedimentos, como por ejemplo *Scrobicularia plana* (Byrne y O'Halloran 1999, Riba *et al.* 2003, 2004a), *Macoma balthica* (Bryan *et al.* 1985, Duquesne *et al.* 2004), *Tapes decussatus* (Mariño-Balsa *et al.* 2003), o *Mya arenaria* (Phelps 1990). La almeja *Tapes semidecussatus*, o *Ruditapes philippinarum* (Reeves 1864) como también se la conoce, es un molusco bivalvo que se entierra en sedimentos blandos y

Introduction

Different species of bivalve mollusks have been identified as suitable indicators of sediment contaminants, especially metals, such as *Scrobicularia plana* (Byrne and O'Halloran 1999, Riba *et al.* 2003, 2004a), *Macoma balthica* (Bryan *et al.* 1985, Duquesne *et al.* 2004), *Tapes decussatus* (Mariño-Balsa *et al.* 2003), and *Mya arenaria* (Phelps 1990). The Manila clam *Tapes semidecussatus*, or *Ruditapes philippinarum* (Reeves 1864) as it is also known, is a soft-sediment dwelling bivalve mollusk that can withstand a wide range of temperature and

que puede soportar un amplio rango de salinidad y temperatura (Carter 2004), por lo presenta la ventaja de poder ser usada para evaluar la toxicidad de sedimentos estuáricos. Además, cumple la mayoría de los criterios establecidos para seleccionar especies para realizar ensayos de toxicidad: está disponible a lo largo de todo el año ya que es una especie comercial en España, es fácil de mantener bajo condiciones de laboratorio, es económicamente relevante y además tiene una amplia distribución geográfica.

El uso potencial de esta especie como organismo de ensayo para la evaluación de la toxicidad de sedimentos ha sido investigado en distintos estudios y bajo distintos diseños de ensayo. Byrne y O'Halloran (1999) estudiaron la mortalidad tras 21 días y el enterramiento después del periodo de exposición. También, Phelps (1990) estudió el enterramiento de los organismos y comparó, por medio del análisis Logit, los efectos de la toxicidad mediante el tiempo efectivo en el que se entierran 50% de los organismos. En España se ha utilizado una modificación de estos dos protocolos para evaluar la calidad de sedimentos contaminados mediante el análisis de la mortalidad y del enterramiento (Riba *et al.* 2004b). Para este ejercicio interlaboratorio se escogió el ensayo con juveniles de *R. philippinarum* y la mortalidad tras 7 y 14 días, así como la actividad de enterramiento en el sedimento problema, como medidas para la caracterización de la toxicidad de materiales de dragado.

Material y métodos

Las almejas fueron obtenidas de un cultivo comercial por cada uno de los laboratorios participantes. Los individuos se recibieron en menos de 24 h y se aclimataron a las condiciones de laboratorio en agua de mar limpia durante al menos dos semanas antes del inicio del ensayo. Durante este periodo los organismos se alimentaron de una mezcla de microalgas (*Tetraselmis chuii*, *Isochrysis galvana* y *Chaetoceros gracilis*) y se mantuvieron en un sistema abierto.

Las condiciones y los parámetros para el desarrollo del ensayo se presentan en la tabla 1. Los sedimentos se añadieron a las cámaras de exposición hasta obtener una profundidad de al menos 5 cm, añadiendo agua de mar en relación 1:3, y se airearon al menos 12 h previamente a la introducción de los organismos. El día de inicio del bioensayo se seleccionaron entre 20 y 40 organismos que fueron introducidos a cada uno de los replicados sucesivamente.

El ensayo subletal finalizó tras 48 h. El número de organismos enterrados en cada uno de los replicados se contabilizó tras 15, 30 y 45 min, y 1, 1.5, 3, 6, 12, 24 y 48 h del inicio de la exposición. Para el cálculo del tiempo estimado en el que se han enterrado 50% de las almejas (TE50) se usó una modificación del análisis Logit. Tras 7 y 14 días se contabilizó el número de organismos vivos mediante el tamizado del sedimento (0.5 mm) y se calculó el porcentaje de mortalidad.

salinity (Carter 2004). Thus, one of the main advantages of this species is its suitability for use in estuarine sediment toxicity bioassays. Moreover, it fulfills many of the criteria established for species selection in toxicity testing: it is available all year round because it is a commercial species in Spain, it is easy to maintain in the laboratory, it is economically relevant and it has a wide geographical distribution.

Its potential use as a test organism in sediment toxicity has been investigated in different studies and under different test designs. Byrne and O'Halloran (1999) studied the mortality after 21 days and the reburrowing activity after different exposure periods. Phelps (1990) studied the burrowing activity and calculated the estimated time for 50% of the clams to burrow by Logit analysis. A modification of these two tests has been used in Spain to study sediment quality using both mortality and burrowing activity as selected endpoints (Riba *et al.* 2004b). For this interlaboratory study we selected the bioassay using juveniles of the bivalve *R. philippinarum*, and mortality after 7 and 14 days and burrowing activity as the test endpoints to characterize dredged material toxicity.

Material and methods

Clams were obtained from a commercial hatchery and received in each laboratory in less than 24 h. Organisms were acclimated to laboratory conditions in clean seawater for at least two weeks before initiating the test. During this period animals were fed a mixture of different species of microalgae (*Tetraselmis chuii*, *Isochrysis galvana* and *Chaetoceros gracilis*) and were maintained in an open-water system.

Test parameters and conditions are summarized in table 1. Sediment was added to the test chambers to obtain a layer of approximately 5 cm and clean seawater was added at a ratio of 1:3. Test chambers were aerated for at least 12 h prior to the addition of the organisms. On initiating the bioassay, 20 to 40 organisms were randomly selected and added to each replicate. The sublethal test finalized after 48 h of exposure. The number of buried organisms in each test chamber was assessed 15, 30 and 45 min, and 1, 1.5, 3, 6, 12, 24 and 48 h after initiating the test. To obtain the estimated time for 50% of the clams to burrow (TE50), a modified Logit analysis was used. After 7 and 14 days the number of living organisms was assessed by sieving the sediment through a 0.5-mm mesh and the percentage of mortality was derived.

To study test reproducibility and interlaboratory variability of the results, the coefficient of variation (CV) was calculated for each sample. This value was obtained by dividing the standard deviation (SD) by the mean value (X):

$$\mathrm{CV}\ (\%) = \frac{\mathrm{SD}}{\mathrm{X}} \cdot 100$$

Moreover, to study the possible effects of this variability on sample classification as toxic or not toxic, the results from each laboratory were studied individually to find differences

Tabla 1. Parámetros y condiciones a seguir para el desarrollo del test con bivalvos juveniles en el laboratorio.
Table 1. Test parameters and conditions to develop the test using juvenile bivalves in the laboratory.

Parameters	Conditions
1. Test type	Static; on whole sediment
2. Temperature	15–20ºC (19ºC recommended)
3. Salinity	36–40
6. Photoperiod	Natural of the season; also continuous light
7. Test chambers	Glass, 10–5 L (aquarium type recommended)
8. Volume of sediment	1.5–2.0 L (1:4 sediment/water)
9. Volume of overlying water	6–8 L (1:4 sediment/water)
10. Water renewal	Not necessary
11. Size and state of organisms	*Ruditapes philippinarum* 1–2 cm diameter; recommended values, they can be higher
12. Number of organisms per chamber	20
13. Number of replicates	2
14. Feeding regime	No
15. Aeration	12 h before introducing the organisms, to ensure dissolved oxygen concentrations equal or higher than 90% of saturation
16. Overlying water	Clean seawater; also artificial
17. Water quality	Daily measurements of temperature; pH, ammonia, salinity and dissolved oxygen measurements at least at the beginning and end of the test
17. Test duration	Lethal, 7–14 days; sublethal, 12 and 48 h
18. Endpoints	50% of organisms burrowed (TE50 h^{-1}, 12–48 h); survival (7–14 days)
19. Test acceptability	TE50 less than 5 h in the negative toxicity control; 90% survival in the negative toxicity control

Para el estudio de la variabilidad interlaboratorio se calculó el coeficiente de varianza (CV) de cada muestra dividiendo la desviación estándar (SD) por el valor de la media de los laboratorios (X):

$$CV\ (\%) = \frac{SD}{X} \cdot 100$$

Además, para el estudio de los posibles efectos de esta variabilidad en la clasificación de las muestras como tóxicas o no tóxicas se estudiaron los resultados de cada laboratorio individualmente para establecer diferencias entre el control de toxicidad negativo (muestra A) y cada una de las muestras de sedimentos analizadas, comparando los resultados obtenidos en los distintos laboratorios. Aunque el número de casos fue suficiente para cada muestra (dos replicados con 40 organismos cada uno), los datos no cumplían las condiciones para la utilización de un análisis estadístico paramétrico, por lo que se aplicó el test de Fisher utilizando el programa informático Simple Interactive Statistical Analysis (SISA; http://home.clara.net/sisa/). Los resultados del enterramiento se estudiaron mediante un ensayo de tipo ANOVA y, en caso necesario, se aplicó el test de Tukey para establecer diferencias entre las respuestas. Se utilizó el programa estadístico SPSS 11.0.

between the negative toxicity control (sample A) and each sample. Although the number of cases was sifficiently high for each sample (two replicates with $n = 40$), the data did not meet the conditions to apply a parametric statistical test, so the Fisher test was applied using the Simple Interactive Statistical Analysis (http://home.clara.net/sisa/). The burrowing activity results were studied using ANOVA and, if necessary, Tukey's test to establish differences in the response of the samples. The SPSS 11.0 software was used.

Results

Laboratories 1 and 2 carried out the bioassay within two weeks after sediment sampling (table 1). Laboratory 3 developed a first test but the results did not meet the acceptance criteria due to high mortality in the negative toxicity control. This laboratory developed a second test but the samples were stored for too long and did not meet the recommended guidelines.

The results obtained for 7 days of exposure do not seem to be sensitive enough for this type of sample: mortality was lower than 5% for all samples and all laboratories, except for sample C (table 2). This sample had the highest mortality values after this exposure period: 28.8%, 41.8% and 100% for

Resultados

Los laboratorios 1 y 2 realizaron el ensayo dentro de las primeras dos semanas tras el muestreo del sedimento (tabla 1). El laboratorio 3 realizó un primer ensayo, pero los resultados no cumplieron el criterio de aceptabilidad debido a la elevada mortalidad registrada con el control de toxicidad negativo. Este laboratorio realizó un segundo ensayo con las muestras, sin embargo, en esta ocasión el tiempo de almacenamiento fue demasiado largo para estar dentro de los rangos recomendados.

Como puede observarse en los resultados de mortalidad a 7 días, ésta no parece una medida lo suficientemente sensible para este tipo de muestras: la mortalidad tras 7 días en todos los laboratotios y para todas las muestras fueron inferiores a 5%, excepto para la muestra C (tabla 2). La muestra C obtuvo valores de 28.8%, 41.8% y 100% en los laboratorios 1, 2 y 3, respectivamente. Con excepción de esta muestra en todos los laboratorios, el análisis estadístico (fig. 1) clasificó todas las muestras como estadísticamente similares al control ($P \leq 0.05$). En cuanto a la mortalidad tras 14 días, ésta alcanzó valores ligeramente superiores para las muestras E y F mientras las muestras B y D mantuvieron mortalidades similares al control negativo. La muestra C aumentó en mortalidad y obtuvo una media de 69.17%. El resultado de los análisis estadísticos mostró diferencias significativas ($P \leq 0.05$) para la muestra C pero también para las muestras E y F. A pesar de esta homogeneidad en la clasificación de las respuestas, la variabilidad de los resultados estimada mediante el CV fue elevada debido a las bajas mortalidades. La muestra C, la única con valores de mortalidad superiores, obtuvo un CV de 66.7% aunque éste se redujo a 38% en los resultados tras 14 días.

laboratories 1, 2 and 3, respectively. The statistical analysis (fig. 1) did not reveal significant differences between any of the samples and the negative toxicity control ($P = 0.05$), except for sample C for all laboratories. When mortality was assessed after 14 days of exposure, higher values were obtained for samples E and F, but samples B and D were similar to the negative toxicity control. Sample C also registered higher mortality, with a mean value of 69.17%. The statistical analysis of the results obtained for 14 days of exposure showed significant differences ($P \leq 0.05$) for sample C, as well as for samples E and F. Even though the sample classification was homogeneous among laboratories, the variability of the results estimated by the CV was high because of the low mortality values. Sample C, the only sample with high mortality values, had a CV of 66.7%, though it decreased to 38% for the 14-day exposure results.

The results of the sublethal endpoint (table 3) showed that laboratory 3 was not comparable to laboratories 1 and 2. The difference in the number of exposed organisms could have influenced test results, since laboratory 3 used 20 organisms per replicate while the other two used 40, and difficulties were encountered in assessing the number of buried organisms due to the fast burrowing activity. The results obtained by the other two laboratories were also very variable and significant differences were only registered for sample C by laboratory 2 (fig. 1).

Discussion

According to these results, mortality seems a suitable endpoint only for samples with high concentrations of metallic compounds (Casado-Martínez *et al.* 2006). Sample C had high

Tabla 2. Resultados de mortalidad tras 7 y 14 días de exposición.
Table 2. Percent mortality after 7 and 14 days of exposure.

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Mortality after 7 d (%)						
Laboratory 1	1.25	0.00	28.8	2.50	3.75	1.25
Laboratory 2	2.50	0.00	41.8	2.50	5.00	3.75
Laboratory 3	0.00	0.00	100.0	0.00	0.00	0.00
SD	1.25	0.00	37.9	1.44	2.60	1.91
Xm	1.25	0.00	56.8	1.67	2.92	1.67
CV %	100.00	0.00	66.7	86.6	89.2	114.00
Mortality afterd 14 d (%)						
Laboratory 1	1.25	0.00	57.5	2.50	10.0	6.25
Laboratory 2	2.50	1.25	50.0	2.50	12.5	7.75
Laboratory 3	0.00	0.00	100.0	0.00	0.00	0.00
SD	1.25	0.72	26.96	1.44	6.61	4.11
Xm	1.25	0.42	69.17	1.67	7.50	4.67
CV %	100.00	173.21	38.98	86.60	88.19	88.08

Tabla 3. Resultados de enterramiento expresados como TE50 (h).
Table 3. Burrowing activity, expressed as TE50 (h).

Laboratory	Replicate	Sample A	Sample B	Sample C	Sample D	Sample E
1	1	0.197	0.021	0.118	0.142	0.052
	2	0.023	0.061	0.476	0.162	0.183
2	1	0.143	0.033	0.873	0.048	0.070
	2	0.166	0.008	0.848	0.162	0.210

El estudio de la medida subletal (tabla 3) muestra que los resultados del laboratorio 3 no fueron comparables con los de los otros laboratorios. El principal factor que pudo haber afectado a esta medida es la diferencia entre el número de organismos expuestos ya que este laboratorio utilizó 20 organismos por replicado y encontró dificultades para contar los enterrados debido a la gran velocidad de enterramiento, según el informe enviado con los resultados. Los resultados en los otros laboratorios fueron también muy variables y sólo podrían considerarse importantes las diferencias registradas para la muestra C en el laboratorio 2 (fig. 1).

Discusión

De acuerdo con los resultados de este ejercicio, la mortalidad parece una medida que sólo es sensible para la evaluación de toxicidad en muestras con altas concentraciones de compuestos metálicos (Casado-Martínez *et al.* 2006). Los resultados para la muestra C, con altas concentraciones de As y Cu y en menor medida Hg, Pb y Zn, son los únicos que mostraronn diferencias al control negativo tras 7 días de exposición. Al aumentar el tiempo de exposición a 14 días aumentó la mortalidad de las muestras E y F, afectadas por contaminación de tipo metálica (Cu, Cd y Hg) y además con concentraciones altas de PCBs. Distintos autores han relacionado la mortalidad en esta especie de bivalvo con la presencia de contaminantes metálicos (Byrne y O'Halloran 2000, Shin *et al.* 2002), especialmente con Cd, Cu, Pb y Zn. Por el contrario, la mortalidad registrada en las muestras afectadas por contaminación de tipo orgánica (muestras B y D) fue similar a la mostrada por el control de toxicidad negativo. No se han encontrado referencias bibliográficas que relacionen directamente la mortalidad de esta especie con la presencia de contaminantes orgánicos, pero estos resultados son similares a los encontrados por Riba *et al.* (2004b), quienes encontraron mortalidades similares a los controles de toxicidad negativos en muestras de sedimentos costeros con concentraciones de Hg y PCBs superiores a los Niveles de Acción 2. De acuerdo con estos resultados, se considera que la letalidad no es una medida sensible para la caracterización de sedimentos afectados únicamente por compuestos de tipo orgánico.

Como ya se ha mencionado anteriormente, los resultados para los distintos laboratorios son homogéneos y la clasificación de las muestras fue similar entre laboratorios. La gran variabilidad interlaboratorio según los altos CV no afecta a la clasificación de las muestras, más bien refleja lo poco concentrations of As and Cu and, to a lesser extent, of Hg, Pb and Zn, and it is the only sample that showed statistically significant differences in comparison to the control sediment after 7 days of exposure. When exposure was increased to 14 days, samples E and F, which had high Cu, Cd, Hg and PCB concentrations, could also be classified as toxic since the mortality values were statistically different from the negative toxicity control. Different authors have related bivalve mortality to metallic compounds (Byrne and O'Halloran 2000, Shin *et al.* 2002), especially Cd, Cu, Pb and Zn. On the other hand, the

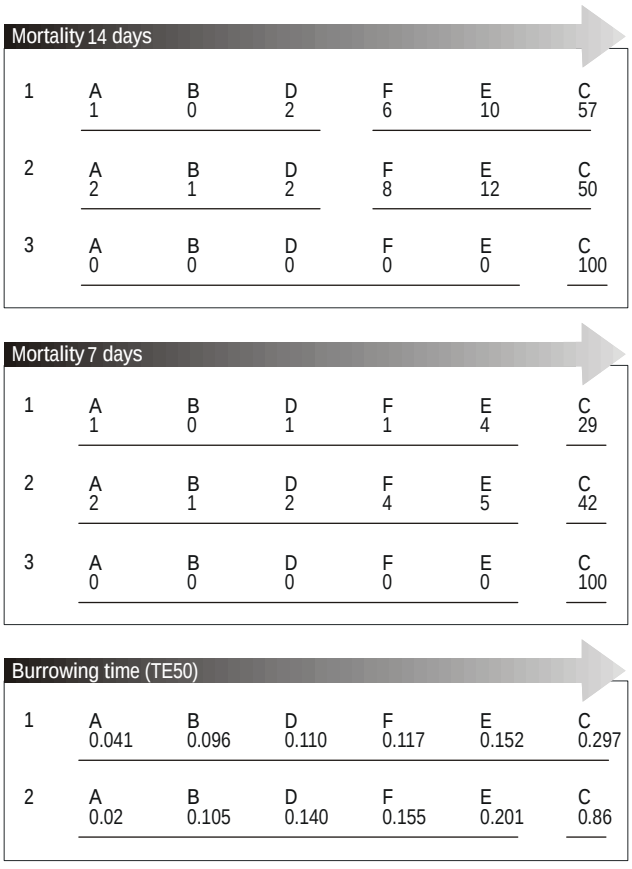

Figura 1. Resultado del análisis estadÌstico de los porcentajes de mortalidad y tiempo de enterramiento (TE50) de almejas. Las muestras subrayadas por una misma línea no son signifativamente diferentes con $P \leq 0.05$ (test de Fisher).

Figure 1. Result of the statistical analysis of mortality percentages and burrowing activity (TE50). Samples underlined by the same line are not significantly different at $P \leq 0.05$ (Fisher test).

adecuado que es este valor para expresar la variabilidad interlaboratorio especialmente para muestras con bajas toxicidades (como en este caso) y si se dispone de un número pequeño de laboratorios. Todos los laboratorios obtuvieron valores de mortalidad bajos para todas las muestras excepto la C: los laboratorios 1 y 2 obtuvieron bajas mortalidades y el laboratorio 3 no registró mortalidad alguna (0%). Al aumentar el tiempo de exposición a 14 días la mortalidad en los dos primeros laboratorios aumentó pero no en el laboratorio 3, lo que hace aumentar los CV de 0–114% tras 7 días a 86.6–173% tras 14 días, aún siendo los resultados homogéneos. En cambio, para la muestra C el aumento de las mortalidades en los dos primeros laboratorios hace descender los CV de 66.7% a 39%. Esta diferencia en los porcentajes de mortalidad, además de influenciar críticamente la noción de variabilidad interlaboratorio ofrecida por los CV, podría deberse a los distintos tiempos de almacenamiento de los sedimentos previamente al ensayo, ya que no se ha identificado ningún otro factor de confusión. El análisis estadístico de los resultados tras 7 días de exposición (fig. 1) clasifica la muestra C como estadísticamente diferente ($P \leq 0.05$) al control negativo de toxicidad aunque tras 14 días de exposición lo son también las muestras E y F según los laboratorios 1 y 2. La diferencia en el tiempo de almacenamiento pudo afectar las muestras con concentraciones intermedias de contaminación, y aunque también era de esperarse una disminución de la toxicidad en la muestra C, éste no fue el caso.

En cuanto a los resultados de enterramiento, éstos han demostrado ser una medida inadecuada, al menos siguiendo el protocolo actual. Se han encontrado efectos adversos en la velocidad de enterramiento de organismos en sedimentos contaminados artificialmente con distintos metales (Roper *et al.* 1995, Shin *et al.* 2002), y diversos autores han utilizado con éxito el enterramiento como medida subletal para evaluar la toxicidad de sedimentos costeros (Byrne y O'Halloran 1999, Riba *et al.* 2004b) bajo distintos protocolos. Existen referencias previas en la literatura en las que se utiliza este ensayo para evaluar muestras de sedimentos de puertos (Byrne y O'Halloran 2000), aunque la medida del enterramiento se ha tomado en sedimento limpio, pero tras 10 y 20 días de exposición a los sedimentos contaminados. Estudios previos de esta medida subletal la han relacionado directamente con la contaminación metálica de las muestras, pero no existen datos para la contaminación de tipo orgánico a excepción de los registrados para sedimentos contaminados con crudo en los que la velocidad de enterramiento ha mostrado una disminución de (Olla y Bejda 1983). Esos resultados parecen confirmar la respuesta registrada para la muestra C, con valores elevados de los componentes metálicos analizados (Casado-Martínez *et al.* 2006), pero no la obtenida con el resto de las muestras, las cuales presentaban contaminación de tipo orgánico. De acuerdo con los resultados obtenidos en este estudio, en este sentido no se consideraría recomendable la medida del enterramiento en los sedimentos al inicio de la exposición. Aunque esta medida subletal pudiera ser una medida eficaz para

mortality recorded for samples B and D, affected by organic contamination, was similar to that found for the negative toxicity control. References could not be found that directly relate organic contamination and bivalve mortality, but our results are similar to those reported by Riba *et al.* (2004b), with mortality values similar to those of negative toxicity controls for coastal sediment samples with Hg and PCB concentrations higher than Action Level 2 (Casado-Martínez *et al.* 2006). Based on these results, it can be concluded that the lethal endpoint for this species is not sensitive enough for sediments affected by organic contamination.

As mentioned before, the results were homogeneous and sample classification was similar for all laboratories. The high interlaboratory variability shown by the CV does not affect sample classification, but rather reflects the inadequacy of this statistic to assess variability when low toxicity values are registered and when only a few laboratories are involved. All laboratories reported low mortality for all samples except C: laboratories 1 and 2 obtained low values, while laboratory 3 reported 0%. When the exposure time increased to 14 days, the mortality values obtained by laboratories 1 and 2 also increased, but laboratory 3 still registered 0%, which caused the CV to increase from 0–114% at 7 days to 86.6–173% at 14 days, even if the results are homogeneous. For sample C, however, the increase in mortalities recorded by laboratories 1 and 2 caused the CV to decrease from 66.7% at 7 days to 39% at 14 days of exposure. This difference in mortality percentages, apart from influencing the interlaboratory variability information provided by the CV, may be related to the different sediment storage time before testing since no other interfering factor could be identified. Statistical analyses only identified as toxic ($P \leq 0.05$) sample C when the test duration was 7 days (fig. 1) and samples E and F when the exposure time was 14 days, according to laboratories 1 and 2. Differences in storage time may affect samples with an intermediate degree of contamination, though a decrease in toxicity for sample C would also have been expected but this was not reported by laboratory 3.

The burrowing activity results indicate the unsuitability of this endpoint following the present test methodology. Adverse effects on burrowing activity have been reported for sediments spiked with different metallic compounds (Roper *et al.* 1995, Shin *et al.* 2002), while burrowing activity has been successfully used for marine and coastal sediment quality assessment (Byrne and O'Halloran 1999, Riba *et al.* 2004b) following different protocols. Moreover, this sublethal endpoint has been used to test sediments from ports (Byrne and O'Halloran 2000), though the endpoint was registered after 10 and 20 days of exposure to contaminated sediments as reburrowing activity in clean sediment. Even though this endpoint seems to be directly affected by certain metallic compounds, the only data available regarding organic pollutants are those reported for oiled sediments (Olla and Bejda 1983). This previous information supports the results obtained for sample C, with high metallic concentrations (Casado-Martínez *et al.* 2006), but

predecir efectos potenciales dado que el enterramiento se considera un método de defensa contra la depredación, si se requiere utilizar en ensayos de toxicidad debería cambiarse el protocolo de ensayo y posiblemente adaptarlo al utilizado por Byrne y O'Halloran (2000).

Aunque este ensayo puede ser utilizado con éxito para la evaluación de la toxicidad de sedimentos costeros afectados por contaminación metálica mediante el uso de la medida letal y subletal, el protocolo aplicado no parece recomendable para la evaluación de la toxicidad de materiales de dragado. La baja sensibilidad de esta especie para clasificar los sedimentos como tóxicos de acuerdo con sus efectos letales, posiblemente debida a la disponibilidad de la contaminación presente a este tipo de organismos filtradores, podría aumentarse mediante la exposición de los organismos durante periodos de tiempo más largos, aunque esto aumentaría la relación coste-eficacia del ensayo. En el caso de la medida subletal, se han encontrado dificultades en cuanto al número de laboratorios capaces de desarrollar con éxito el ensayo. De acuerdo con estos resultados, pareció inadecuado considerar valores límites de toxicidad para la clasificación de materiales de dragado. En cualquier caso, y si el laboratorio lo cree conveniente, este ensayo puede ser incluido como parte de una serie más amplia de estudios para la caracterización de dragados portuarios ya que ofrece información útil y complementaria a otros bioensayos.

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cannot confirm the lethal or sublethal effects related to organic contamination. In this sense our results indicate that burrowing activity measured just after the addition of test organisms is not a suitable endpoint for dredged material characterization. Although this endpoint introduces useful information, since burrowing is considered a defense mechanism against predation, the results suggest that the test design should be changed or possibly adapted to a new one such as that used by Byrne and O'Halloran (2000).

Despite previous successful results obtained for sediment toxicity assessment when testing samples affected by metallic contamination, this protocol does not seem to be suitable for dredged material toxicity assessment. The lethal endpoint is not sensitive enough to classify sediment toxicity probably because of the low availability of contaminants for filter-feeding species such as the Manila clam. Although the sensitivity can be increased by increasing the test duration, this would reduce the test's cost effectiveness. In the case of the sublethal endpoint, difficulties were found related to the number of laboratories capable of developing the test successfully. Based on these results, no limit values for dredged material classification were considered. Nevertheless, this assay could be included as part of a wider test battery since it can provide additional useful information for dredged material management.

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Nota de Investigación/Research Note

Ejercicio interlaboratorio de bioensayos marinos para la evaluación de la calidad ambiental de sedimentos costeros. VI. Análisis general de resultados y conclusiones del ejercicio

Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. VI. General analysis of results and conclusions of the exercise

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Resumen

Para estudiar si los bioensayos de toxicidad cumplen ciertos criterios a nivel nacional se realizó un ejercicio interlaboratorio diseñado principalmente para estudiar el desarrollo, la optimización y la validación de los métodos disponibles para la evaluación de la toxicidad en material de dragado. Se evaluó la eficiencia y la utilidad de este tipo de técnicas y se compararon los resultados de toxicidad obtenidos con la caracterización química tradicional. Este ejercicio incluyó dos fases y mostró resultados satisfactorios especialmente para el ensayo Microtox®, el ensayo con el desarrollo embrionario del erizo de mar y el ensayo con anfípodos. Estos tres ensayos parecen factibles y ofrecen información útil para la gestión de dragados portuarios. Los resultados del análisis multivariante realizado para unir la toxicidad y la contaminación registrada con los ensayos mostraron que las respuestas tóxicas registradas están correlacionadas en mayor o menor medida con los distintos contaminantes analizados en el sedimento.

Palabras clave: ecotoxicología, toxicidad de sedimento, análisis multivariate, guías de calidad de sedimento, gestión de material de dragado.

Abstract

To ensure that toxicity bioassays comply with national criteria, an interlaboratory study was carried out that focussed mainly on the development, optimization and validation of methods for dredged material toxicity assessment. The performance of these techniques was assessed in a validation study and the results were compared with the traditional chemical characterization. This study included two phases and showed promising results in particular for the Microtox® bioassay, the bioassay using crustacean amphipods and the embryo-larval development bioassay using sea urchins. These tests seem feasible and give useful information for dredged material management. Moreover, to evaluate sediment quality, the chemical concentration of the contaminants of concern and toxicity test endpoints were linked using multivariate analysis. The results of this analysis showed that the toxic responses were correlated to different contaminants measured in the sediments.

Key words: ecotoxicology, sediment toxicity, multivariate analysis, sediment quality guidelines, dredged material management.

Introducción

Para evaluar la variabilidad en cuatro bioensayos se llevó a cabo un ejercicio interlaboratorio como parte del proceso de desarrollo y selección de una batería de bioensayos para la caracterización de materiales de dragado en España. Los ensayos seleccionados fueron el Microtox®, siguiendo el protocolo para la fase sólida (SPT) y el nuevo protocolo básico para muestras sólidas (BSPT); el ensayo de desarrollo larvario a 48 h con embriones de erizo de mar para la evaluación de lixiviados de sedimentos; y el bioensayo con anfípodos y juveniles de bivalvo, ambos desarrollados sobre la fase sólida. Los principales objetivos de estudio de los ensayos fueron: (1) evaluar la precisión de cada bioensayo para caracterizar la toxicidad de

Introduction

An interlaboratory study was carried out to evaluate the variability related to the execution of bioassays by different laboratories as part of the development and selection of a battery of tests suitable for dredged material characterization processes in Spain. The selected bioassays included the Microtox®, following the solid-phase test (SPT) and the new basic solid-phase test (BSPT) protocols; the 48-h sea urchin embryo-larval development bioassay to test elutriates; and the bioassay using amphipods and the burrowing and survival bioassay using juvenile bivalves, both developed on the whole sediment. The main objectives of the study were: (1) to assess the precision of each of the bioassays to characterize dredged

materiales de dragado cuando son desarrollados por distintos laboratorios; (2) identificar los principales factores de confusión que pueden afectar la interpretación de los resultados; (3) discutir las posibles causas de variabilidad interlaboratorio para mejorar el protocolo estándar y así el uso de los bioensayos como herramienta complementaria para la caracterización y gestión de dragados portuarios; y (4) discutir las guías biológicas previamente propuestas para cada ensayo para considerar las muestras como tóxicas o no tóxicas (Casado-Martínez *et al.* 2006a).

Las condiciones relacionadas con el muestreo y manipulación de los sedimentos han sido previamente discutidas (Casado-Martínez *et al.* 2006a), así como los resultados para cada uno de los bioensayos utilizados (Casado-Martínez *et al.* 2006b–e). El objetivo de este trabajo es analizar globalmente los resultados y conclusiones, y estudiar el uso de los ensayos biológicos en los procesos de caracterización para la gestión de dragados en España conjuntamente con la tradicional caracterización química. Además, las series de datos se estudiaron mediante un análisis estadístico multivariante para evaluar la relación entre los efectos biológicos adversos y la concentración de los compuestos potencialmente tóxicos .

Material y métodos

Con el propósito de encontrar posibles relaciones entre las distintas variables se utilizó un análisis factorial de componentes principales como procedimiento de extracción (DelValls y Chapman 1998, Riba *et al.* 2003). Los datos obtenidos se analizaron individualmente para cada fase del ejercicio mediante la técnica de extracción de componentes principales con el programa estadístico Statistica® 5.0. El análisis de los factores se realizó en la matriz de correlación y todos los datos biológicos fueron tratados de forma similar suponiendo igual importancia para cada ensayo. Los datos de toxicidad se transformaron de forma que mostrasen un aumento en el valor al aumentar los efectos biológicos.

Resultados

Fase I

El conjunto de variables originales comprendió 28 series de datos, cada una con 10 variables químicas (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, Σ_7-PCBs y Σ_{16}-PAHs), 3 parámetros convencionales del sedimento (porcentaje de arenas, porcentaje de finos y contenido en material orgánica) y 15 variables de efecto biológico: los resultados para el ensayo Microtox® SPT expresados como unidades tóxicas (100/EC50), el porcentaje de larvas anormales tras 48 h de exposición a los lixiviados, el porcentaje de mortalidad de anfípodos, así como el porcentaje de mortalidad de bivalvos tras 14 días de exposición (tabla 1). Sólo se incluyeron en el análisis multivariante los resultados de los laboratorios que obtuvieron resultados con todas las muestras y que, además, se consideraron aceptables: cinco laboratorios para el Microtox®, nombrados A1, A2, A3, A4 y

material toxicity when developed by different operators and laboratories, (2) to identify the main interfering factors affecting the test results and their interpretation, (3) to discuss the possible causes of interlaboratory variation in order to improve the standard protocol and the use of bioassays as a complementary tool for dredged material characterization and management, and (4) to support biological guidelines previously derived or proposed for each test to consider whether a sample is toxic or not toxic.

The sediment sampling and handling conditions are described in Casado-Martínez *et al.* (2006a), and the results for each bioassay have been discussed individually (Casado-Martínez *et al.* 2006b–e). The objective of this paper is to give an overview of the interlaboratory variability when using sediment bioassays and to study the performance of such tests for dredged material characterization. Furthermore, the data sets have been used to study the relationship between the adverse biological effects and the concentration of potentially toxic substances present in the environment by means of multivariate statistical techniques.

Material and methods

A principal component extraction followed by multivariate analysis was used to link the chemical quantification and the toxicological responses registered by each laboratory and for each bioassay (DelValls and Chapman 1998, Riba *et al.* 2003). The data sets for each phase were analyzed separately using the factor analysis option of the Statistica® 5.0 program, using the principal components technique as the extraction procedure. The factor analysis was performed on the correlation matrix and all biological data were treated similarly assuming that the significance of each test was equal. Moreover, the toxic responses were transformed to show an increase with biological damage.

Results

Phase I

The original data set comprised 28 variables that included 10 chemical variables (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, Σ_7-PCBs and Σ_{16}-PAHs), 3 conventional parameters of the sediment (percentage of sand, percentage of fine sediments and organic matter content), and 15 biological effect variables: the results of the Microtox® SPT expressed as toxic units (100/EC50), the percentage of abnormal sea urchin larvae after 48 h of exposure to the elutriates, the percentage of amphipod mortality and the percentage of bivalve mortality after 14 days of exposure (table 1). Only those laboratories that reported results for all samples and those considered for each bioassay (as discussed previously) were included for the multivariate exploratory analysis: five laboratories for the Microtox® bioassay, named A1, A2, A3, A4 and A5 (Casado-Martínez *et al.* 2006b); four for the amphipod bioassay, named B1, B3, B4 and B5 (Casado-Martínez *et al.* 2006c); two for the bioassay using

Tabla 1. Valores de las 28 variables de la fase I usadas en el análisis multivariante. Las medidas de toxicidad son A# = 100/EC50, B# = % de mortalidad de anfípodos, C# = % mortalidad de bivalvos, D# = % de larvas anormalmente desarrolladas; # son los códigos asignados previamente a cada laboratorio.
Table 1. Values of the 28 variables from Phase I used in the multivariate analysis. Toxicity endpoints are A# = 100/EC50, B# = percent amphipod mortality, C# = percent bivalve mortality, and D# = percent of abnormal sea urchin larvae; # indicates the code number assigned to each laboratory.

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
% sand	99.8	40.4	9.60	20.3	39.9	38.2
% fine sediment	0.04	59.5	90.2	77.3	58.7	57.8
% OM	1.07	13.8	10.6	14.8	17.6	10.6
As	3.42	30.8	531	67.3	29.0	102
Cd	0.92	1.32	2.50	2.00	2.88	98.5
Cr	0.10	14.9	24.1	18.3	93.9	66.6
Cu	6.98	203	1497	103	601	666
Hg	0.05	1.98	1.99	0.74	4.12	136
Ni	0.06	20.1	7.10	26.3	32.3	29.0
Pb	2.28	86.9	384	148	455	1397
Zn	21.3	378	1857	476	1165	8651
Σ_7-PCBs	0.001	145	0.001	112	273	123
Σ_{16}-PAHs	0.001	0.001	0.001	66.7	1.80	0.91
A1	0.003	0.76	0.13	0.20	0.28	0.30
A2	0.003	0.68	0.21	0.18	0.23	0.25
A3	0.02	0.38	0.11	1.01	0.24	0.54
A4	0.002	0.17	0.16	0.14	0.03	0.14
A5	0.02	0.61	0.67	0.63	0.20	0.25
B1	12.5	75.0	100	67.5	57.5	100
B3	1.60	47.3	90.0	30.0	35	100
B4	3.33	45.0	81.7	43.3	41.67	100
B5	15.0	53.3	61.7	60.0	73.33	80.0
C1	1.25	0.00	57.5	2.50	10	6.25
C2	2.50	1.25	50.0	2.50	12.5	7.75
D2	100	100	85.3	55.0	49.7	56.3
D3	31.5	20.0	82.3	10.5	35.4	100
D4	83.5	94.4	100	8.00	7.00	5.50
D5	65.5	88.0	89.3	13.3	24.0	13.0

A5 (Casado-Martínez *et al.* 2006b); cuatro para el ensayo con anfípodos, identificados como B1, B3, B4 y B5 (Casado-Martínez *et al.* 2006c); dos para el ensayo con bivalvos, C1 y C2 (Casado-Martínez *et al.* 2006d); y cuatro para el ensayo con larvas de erizo de mar, D2, D3, D4 y D5 (Casado-Martínez *et al.* 2006e).

Los resultados del análisis agruparon los datos originales en tres nuevos factores que explicaban 80% de la varianza original. Estos nuevos factores van a ser descritos según las variables dominantes con un peso positivo de 0.25 o superior (tabla 2) aunque los valores negativos en los nuevos factores son de igual importancia. El primer factor, que suma 35% de la varianza total, combina el porcentaje de finos y los compuestos

bivalves, named C1 and C2 (Casado-Martínez *et al.* 2006d); and four for the bioassay using sea urchin larvae, named D2, D3, D4 and D5 (Casado-Martínez *et al.* 2006e).

Analysis of the original data revealed three new factors that accounted for more than 80% of the total original variance. These new factors will be described according to the dominant variables with a minimum loading of 0.25 (table 2). The first extracted factor, which accounted for more than 35% of the total variance, combined the percentage of fine sediments and the chemical compounds As and Cu, as well as Pb and Zn, and Ni, PCBs and PAHs with negatives loadings. The biological effect variables that reported positive loadings in this factor comprised the results of laboratories 4 and 5 for the Microtox®

As y Cu así como el Pb y Zn, y los compuestos Ni, PCBs y PAHs con pesos negativos. Las variables biológicas con pesos positivos para este factor incluyen los resultados del Microtox® en los laboratorios 4 y 5, los resultados de los ensayos con anfípodos y con almejas y todos los resultados del ensayo con embriones de erizo de mar excepto los correspondientes al laboratorio 2. El segundo factor explica 20% de la varianza total y aparece relacionado con el contenido de ciertos metales (Cd, Cr, Hg, Ni, Pb y Zn obtienen pesos positivos mayores a 0.6) y el efecto tóxico registrado para el ensayo de anfípodos, los resultados de un laboratorio con el Microtox® y los resultados de otro laboratorio con el ensayo de desarrollo larvario. Por último, el tercer factor extraído, que explica 18% de la varianza, representa el porcentaje de finos en el sedimento y el contenido en materia orgánica. Las variables químicas Ni, PCBs y PAHs y los efectos registrados con el ensayo Microtox® obtuvieron los mayores pesos en cada caso para este factor.

Los resultados de este análisis se presentan gráficamente en la figura 1, que representa la contribución de los factores en cada caso de estudio (muestras). Al parecer, los compuestos químicos agrupados en los factores 1 y 2 son los causantes de los efectos letales registrados, con una importante contribución de este factor en las muestras C, E y F, aunque es difícil identificar los compuestos causantes de esta toxicidad ya que las respuestas tóxicas no se agrupan uniformemente en ninguno de los nuevos factores. Los efectos letales registrados mediante el ensayo con almejas en los dos laboratorios tienen pesos positivos alrededor de 0.9 para el factor 1 y con una importante contribución de la muestra C. Esta medida tóxica parece reproducible entre los laboratorios y los efectos pueden relacionarse principalmente con las concentraciones de As y Cu y en menor medida de Pb y Zn. Para el ensayo con anfípodos los valores más altos se localizan también en el factor 1 aunque con pesos positivos en todos los nuevos factores extraídos: parece que la mortalidad de anfípodos se ve afectada principalmente por las especies metálicas As, Cu, Cd, Cr, Hg, Ni, Pb y Zn pero también por los compuestos orgánicos analizados. En cualquier caso los resultados parecen reproducibles entre laboratorios aunque se encuentran ligeras diferencias para el laboratorio 5, que utilizó la especie *Mycrodeutopus gryllotalpa* y obtuvo pesos más elevados para el factor 3, donde se encuentran los mayores pesos para el porcentaje de finos, el contenido en materia orgánica pero también de los compuestos de naturaleza orgánica.

Los resultados del ensayo Microtox® obtuvieron pesos positivos para el factor 3, aunque los laboratorios 4 y 5 tuvieron pesos positivos también para el factor 1. Los resultados del análisis multivariante muestran que los resultados son reproducibles entre los laboratorios ya que en todos los casos se han obtenido pesos positivos elevados en el factor 3 y por lo tanto las respuestas tóxicas estarían relacionadas principalmente a las variables incluidas en este factor (porcentaje de finos y materia orgánica en el sedimento y los compuestos químicos Ni, PCBs y PAHs).

Tabla 2. Peso de los tres factores principales para cada una de las 28 variables. Sólo aquellos pesos superiores a 0.25 están incluidos. Las medidas de toxicidad son A# = 100/EC50, B# = % de mortalidad de anfípodos, C# = % mortalidad de bivalvos, D#=% de larvas anormalmente desarrolladas; # son los códigos asignados previamente a cada laboratorio.

Table 2. Sorted rotated factor loadings of 28 variables in the three principal factors. Only loadings greater than 0.25 are included. Toxicity endpoints are A# = 100/EC50, B# = percent amphipod mortality, C# = percent bivalve mortality, and D# = percent of abnormal sea urchin larvae; # indicates the code number assigned to each laboratory.

	Factor 1 (37.85%)	Factor 2 (25.39%)	Factor 3 (18.08)
% sand	–0.602	–	–0.752
% fine sediment	0.616	–	0.751
OM	–	–	0.904
As	0.948	–	–
Cd	–	0.896	–
Cr	–	0.752	–
Cu	0.908	–	–
Hg	–	0.897	–
Ni	–0.266	0.636	0.700
Pb	0.264	0.953	–
Zn	0.275	0.907	–
Σ_7-PCBs	–0.423	0.395	0.568
Σ_{16}-PAHs	–0.314	–	0.563
A1	–	–	0.578
A2	–	–	0.580
A3	–0.261	0.263	0.709
A4	0.598	–	0.581
A5	0.573	–0.335	0.704
B1	0.736	0.439	0.483
B3	0.790	0.562	–
B4	0.701	0.649	0.251
B5	0.323	0.695	0.610
C1	0.904	–	–
C2	0.894	–	–
D2	–	–0.721	–0.442
D3	0.680	0.641	–0.351
D4	0.449	–0.744	–0.348
D5	0.490	–0.728	–

bioassay, all the results of the bioassay using amphipods and juvenile clams, and all but the laboratory 2 results for the bioassay using sea urchin larvae. The second factor explained 20% of the total variance and is related to the metal content (Cd, Cr, Hg, Ni, Pb and Zn had positive loadings higher than 0.6) and the toxic effects recorded for the amphipod bioassay, the results reported by one laboratory for the Microtox®

Los resultados del ensayo sobre la fase líquida mostraron en la Fase I una respuesta más variable entre laboratorios: el laboratorio 2 no mostró factores con pesos positivos en ninguno de los nuevos factores y por lo tanto no se podría establecer ningún tipo de relación entre las respuestas tóxicas y las variables fisicoquímicas. Las diferencias en los resultados han sido previamente discutidas (Casado-Martínez *et al.* 2006e) y pudieron estar influenciadas por otros factores relacionados con la preparación de las muestras y no incluidos en el análisis multivariante. En cualquier caso las muestras se distribuyeron desde un mismo laboratorio al mismo tiempo y posibles cambios en las propiedades químicas del sedimento pudieron haberse debido a cambios durante el almacenamiento, extracción o pre-tratamiento de las muestras. Los efectos registrados para los otros tres laboratorios parecen debidos a las variables explicadas por el factor 1 y, por lo tanto, el desarrollo anormal de las larvas aparecería relacionado con las especies químicas As, Cu, Pb y Zn aunque para un laboratorio en particular también se relacionan con las concentraciones de Cd, Cr, Hg, Ni, y PCBs. Los pesos negativos tan elevados en el factor 3 parecen indicar que los posibles efectos no sólo se relacionan con los compuestos metálicos sino que cuestionan los posibles efectos causados por la presencia de compuestos orgánicos en los lixiviados.

Fase II

El conjunto de datos originales comprendía 13 variables relacionadas con las propiedades del sedimento (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, Σ_7-PCBs y Σ_{16}-PAHs y el porcentaje de arenas, porcentaje de finos y contenido en material orgánica del sedimento) y 14 variables de efectos biológicos (tabla 3): bioassay and by another for the bioassay using sea urchin larvae. The third factor, which accounted for more than 18% of the total variance, represented the fine particles of the sediment and the organic matter content. The chemical variables Ni, PCBs and PAHs and the effects recorded using the Microtox® bioassay obtained their highest loadings for this factor.

Figure 1 shows the factor scores estimated for each of the six cases, which quantify the prevalence of every factor for each sample used in the interlaboratory study. Apparently the chemical compounds grouped in factors 1 and 2 are responsible for the lethal effects registered (samples C, E and F have positive factor scores), but it is difficult to clearly identify the toxicity-causing compounds since the toxic responses are not grouped uniformly in any of the new factors. In the case of the lethal effects registered for the juvenile clams, the two participating laboratories had positive loadings of around 0.9 in factor 1, which are explained by the high positive factor score for sample C. Moreover, it seems highly reproducible between laboratories and the effects are mainly related to the As and Cu concentrations, and to a lesser extend to Pb and Zn. For the bioassay using amphipods, the highest values were found in factor 1, although positive loadings were found in all the new extracted factors: it seems that amphipod mortality is primarily affected by the metallic compounds As, Cu, Cd, Cr, Hg, Ni, Pb and Zn, as well as by organic compounds. Nevertheless, the results seem reproducible between laboratories and only slight differences were found for laboratory 5, which used a different amphipod species (*Mycrodeutopus gryllotalpa)* and obtained a higher loading for factor 3, related not only to the fine-grain size and organic matter content, but also to organic compounds.

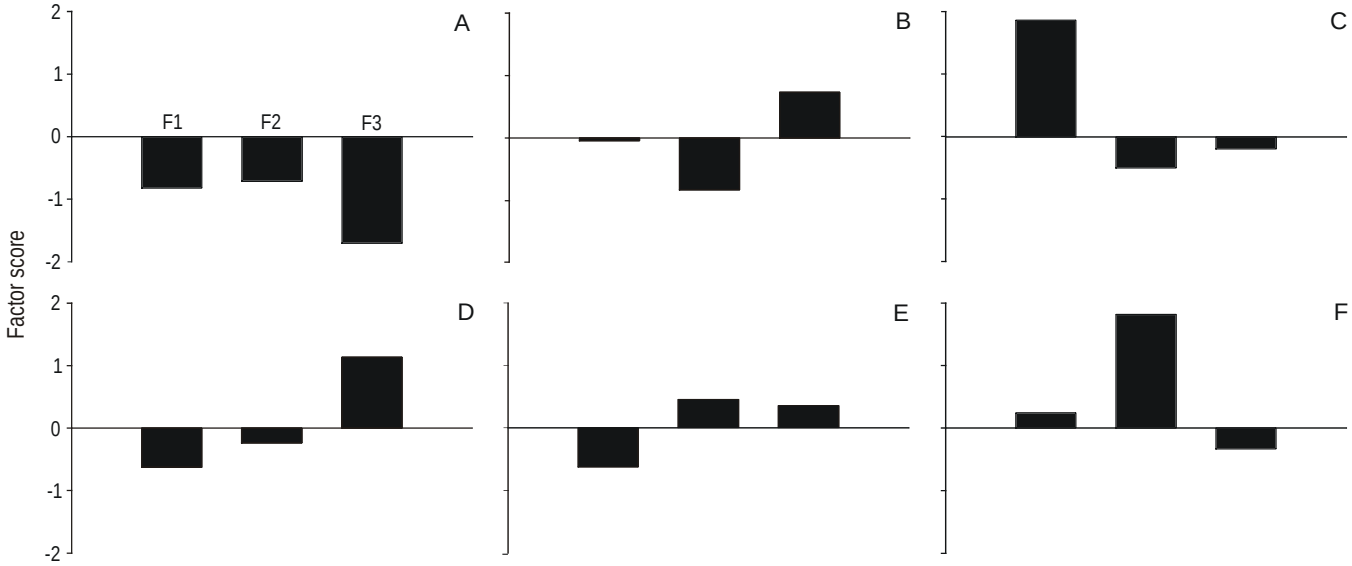

Figura 1. Contribución de los factores estimados para cada uno de los seis casos de la Fase I (A, B, C, D, E y F) al centroide de todos los casos para los datos originales.
Figure 1. Factor scores estimated for each of the six Phase I cases (A, B, C, D, E and F) relative to the centroid of all cases from the original data.

siete laboratorios para el ensayo Microtox® siguiendo el protocolo SPT (A1 a A7), tres siguiendo el protocolo BSPT (B1 a B3) y cuatro para el ensayo de desarrollo larvario con embriones de erizo de mar (C1 a C4). Los resultados clasifican las variables originales en cinco nuevos factores que explican el 100% de la varianza total. La tabla 4 incluye los porcentajes de varianza explicada por cada uno de los nuevos factores y los pesos de cada variable original. En la figura 2 los coeficientes de los nuevos factores para cada uno de los casos (muestras) se representan frente al centroide de todos los casos de los datos originales para estimar la contribución de cada uno de los casos en las nuevas variables o factores. El primer factor, que explica más del 45% de la varianza total, representa las variables contenido en materia orgánica, Cd, Cr y Pb, con los pesos más elevados para las concentraciones de PCBs y PAHs. Este factor también explica la mayor parte de la variabilidad asociada al Microtox® con ambos protocolos (SPT y BSPT), y a los efectos del ensayo con embriones de erizo de mar de dos laboratorios. El factor 2 representa el porcentaje de finos y el contenido en materia orgánica del sedimento, aunque para esta última variable con pesos inferiores a los encontrados para el factor 1 y las especies metálicos As, Cr y Hg. Este factor explica la mayor parte de la variabilidad asociada con el ensayo con embriones de erizo de mar, que también parece influido por el Ni, representado por el factor 5 y con altos pesos positivos. El factor 3, que explica algo más del 15% de la varianza total, explica parte de la varianza asociada al As, Cu, Pb y Zn y a uno de los laboratorios para el Microtox®. Los otros dos factores (4 y 5) explican cada uno algo menos del 10% de la varianza total pero con correlaciones interesantes para algunas de las variables. El factor 4 agrupa el contenido en material orgánica de los sedimentos con los compuestos Hg y PCBs, así como los efectos tóxicos registrados en la mitad de los laboratorios que realizaron el Microtox®. Por otro lado, el factor 5 agrupa las concentraciones de Ni, los efectos tóxicos registrados con el ensayo con embriones de erizo de mar y, en menor medida, ciertos efectos en la disminución de la luminiscencia.

Aunque los resultados obtenidos en la Fase II son similares a los obtenidos en la Fase I la reproducibilidad de las respuestas tóxicas parece mucho más conservativa, especialmente con relación al nuevo protocolo del Microtox® (BSPT). El análisis multivariante ha identificado una mayor correlación entre Hg, PCBs y el contenido en materia orgánica en el factor 4, que también explica parte de la varianza de los resultados tóxicos con este protocolo. Por otro lado la contribución de ciertos compuestos metálicos en las respuestas tóxicas del ensayo de embriones de erizo de mar, como por ejemplo las de As, Cr, Pb y Zn, aparecen en el análisis de los resultados de ambas fases aunque la Fase II identifica también los compuestos Hg y Ni.

Discusión

Los resultados del ejercicio interlaboratorio con el ensayo Microtox® y el ensayo con anfípodos parecen ofrecer resultados satisfactorios con relación a las condiciones de desarrollo y en especial incluyendo las circunstancias

The toxic responses measured by the Microtox® device had positive loadings in factor 3, although laboratories 4 and 5 reported positive loadings also for factor 1. The results of the multivariate analysis show a reproducible effect between laboratories represented by the high positive loadings of the toxicity variables in factor 3 and thus the toxic response registered by the Microtox® system can be described principally by the contribution of the variables explained by this factor (this factor includes the percentage of fine particles and organic matter content in the sediments, as well as the chemical compounds Ni, PCBs and PAHs).

The results of the only liquid-phase bioassay conducted (the bioassay using sea urchin embryos) showed a more variable response among laboratories in Phase I of the exercise: laboratory 2 did not show positive loadings for any of the extracted factors and no relationship can therefore be elucidated between the measured variables and the toxic effects. The differences in the results have been previously identified (Casado-Martínez *et al.* 2006e) and could be influenced by different interfering factors during sample manipulation. Samples were distributed from the coordinator laboratory and changes in the sediment chemistry could be related to changes during the storage, elutriation and pretreatment of the samples. Factor 1 seems to explain some of the effects registered by three laboratories; thus, the abnormal development of the sea urchin embryos would be related to As, Cu, Pb and Zn although the response for one laboratory is also related to other compounds (Cd, Cr, Hg, Ni, and PCBs). The high negative loadings in factor 3 suggest that the effects on the normal development are not only related to the metallic compounds cited before, but also question the effects caused by the presence of certain organic compounds.

Phase II

The original data set comprised 13 variables related to the sediment properties (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, Σ_7-PCBs and Σ_{16}-PAHs, and percentage of sand, percentage of fine sediments and organic matter content) and 14 biological effect variables (table 3), including seven laboratories that reported results for the Microtox® SPT protocol (A1 to A7), three sets of results for the BSPT protocol (B1 to B3) and four sets of results for the bioassay using sea urchin embryos (C1 to C4). The results classified the original variables into five new factors that explained 100% of the total variance. Table 4 includes the percentage of explained variance for the new extracted factors and the factor loadings for each original variable. Figure 2 shows the factor scores estimated for each case plotted to the centroid of all cases for the original data to estimate the contribution of each case. Factor 1, which accounted for more than 45% of the total variance, represents the organic matter content and the chemical compounds Cd, Cr, Pb, PCBs and PAHs. This factor also accounted for most of the variability related to the Microtox® bioassay, for both the SPT and BSPT protocols, and for the effects of the bioassay using sea urchin larvae registered

Tabla 3. Valores para cada una de las 27 variables de las segunda fase del estudio usadas para el análisis multivariante. Las medidas de toxicidad son A#=100/EC50 SPT; B#= 100/EC50 BSPT; C#= % de larvas anormalmente desarrolladas. # son los códigos asignados previamente a cada laboratorio.
Table 3. Values of the 27 variables from Phase II used in the multivariate analysis. Toxicity endpoints are A# = 100/EC50 SPT, B# = 100/EC50 BSPT, and C# = percent of abnormal sea urchin larvae; # indicates the code number assigned to each laboratory.

	Sample A'	Sample B'	Sample C'	Sample D'	Sample E'	Sample F'
% sand	99.0	42.9	56.0	8.36	5.00	14.5
% fine sediment	0.68	57.1	44.0	91.4	94.0	47.4
% OM	1.50	14.0	6.30	9.37	9.00	15.1
As	3.98	33.7	273	336	109	104
Cd	0.87	2.00	1.32	1.03	0.17	2.00
Cr	0.22	16.3	8.13	12.1	38.2	23.1
Cu	8.67	198	772	497	5.01	204
Hg	0.09	1.54	1.20	0.90	1.90	1.43
Ni	0.07	23.9	129	6.18	74.7	32.0
Pb	3.98	97.4	218	211	21.6	286
Zn	24.5	405	1176	974	138	778
Σ_7-PCBs	n.d.	130	0.00	2.29	109	256
Σ_{16}-PAHs	n.d.	n.d.	0.00	0.00	4.21	13.9
A1	0.0013	0.13	0.22	0.08	0.16	0.63
A2	0.0049	0.24	0.32	0.17	0.35	1.28
A3	0.0014	0.25	0.30	0.09	0.21	0.56
A4	0.0001	0.24	0.15	0.04	0.06	0.12
A5	0.0004	0.13	0.28	0.05	0.11	0.29
A6	0.01	0.35	0.30	0.10	0.38	1.47
A7	0.01	0.48	0.42	0.02	0.58	0.45
B1	0.003	0.29	0.36	0.02	0.37	1.66
B2	0.0001	0.25	0.46	0.16	0.39	1.59
B3	0.01	0.34	0.56	0.39	0.76	2.05
C1	18.00	13.00	64.00	93.00	99.0	40.00
C2	9.00	4.00	21.00	8.00	76.0	22.00
C3	47.00	29.00	88.00	53.00	100.00	91.00
C4	7.00	9.00	20.00	30.00	100.00	81.00

particulares de cada laboratorio. El ensayo con almejas no ofreció los resultados esperados posiblemente debido a los pocos de laboratorios que participaron en el estudio; sin embargo, este ensayo parece factible para la evaluación de la toxicidad de sedimentos. Se requiere un mayor desarrollo de los protocolos para las medidas letal y subletal, para poder ser usadas en la gestión de material de dragado. Del mismo modo la sensibilidad de esta especie parece menor a la de las especies de anfípodos utilizadas, que parecen más recomendables para la caracterización de este tipo de materiales durante su gestión. El ejercicio interlaboratorio se repitió para el ensayo con larvas de erizo y para el ensayo Microtox®, ya que los resultados obtenidos inicialmente no fueron tan contundentes como se

by two of the laboratories. Factor 2 represents the percentage of fine sediments and the organic matter content (although the factor loading is lower than for factor 1), as well as the metallic compounds As, Cr and Hg. This factor also explains most of the variability related to the sea urchin embryo assay, which was also affected by Ni, represented in factor 5 and with high positive loadings. Factor 3, which accounted for more than 15% of the total variance, is related to As, Cu, Pb and Zn, as well as to the toxic response registered by one of the laboratories using the Microtox® device. Factors 4 and 5 each accounted for less than 10% of the total variance, but interesting correlations were found. Factor 4 groups the organic matter content of the sediments and the Hg and PCB concentrations

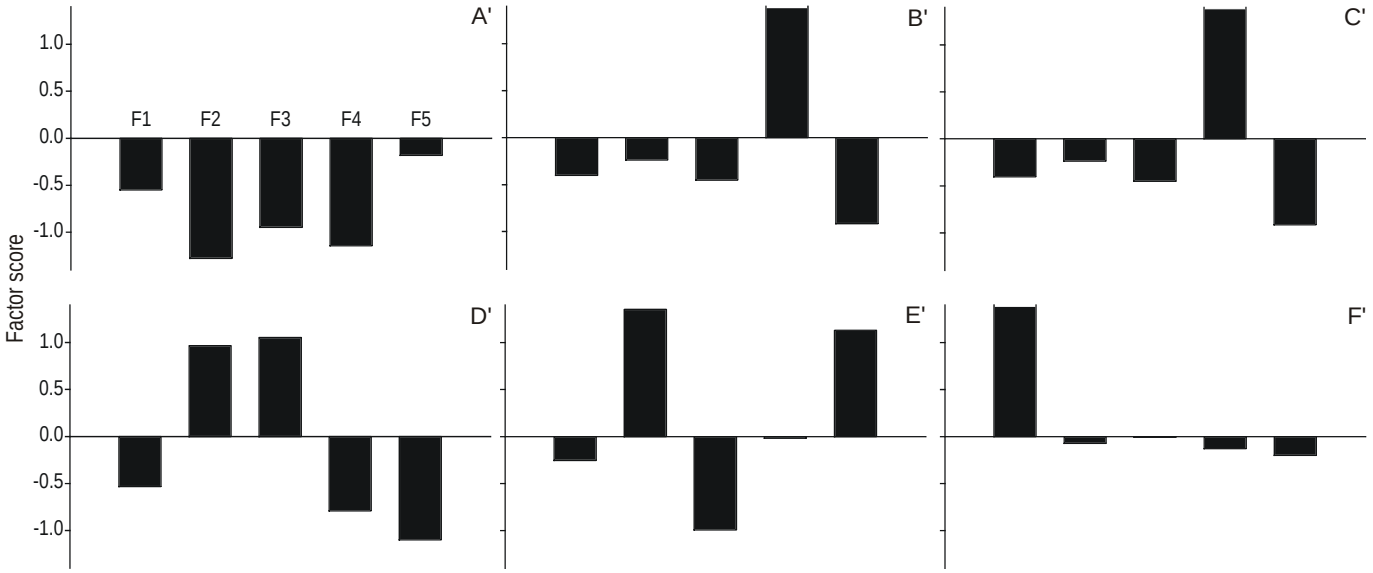

Figura 2. Contribución de los factores estimados para cada uno de los seis casos de la fase II (A', B', C', D', E' y F') al centroide de todos los casos para los datos originales.
Figure 2. Factor scores estimated for each of the six Phase II cases (A', B', C', D', E' and F') relative to the centroid of all cases from the original data.

esperaban. La presencia de algunos factores de confusión a la hora de interpretar los resultados de la Fase I se evitó en la Fase II. Tras el segundo ejercicio la variabilidad interlaboratorio disminuyó hasta encontrarse en rangos similares a los de estudios previos. La homogeneidad de los protocolos para asegurar la compatibilidad de los resultados obtenidos por los diferentes operadores indica que los bioensayos sobre sedimento parecen recomendables. Si los resultados van a ser utilizados con fines de regulación ambiental, el personal debería estar convenientemente formado y sería recomendable su participación en ejercicios y cursos de evaluación continuada, lo que permitiría la evaluación de la variabilidad inter e intralaboratorio para cada uno de los bioensayos que se utilizan en la gestión del material de dragado en España.

Es difícil interpretar los resultados de los ensayos de toxicidad con relación a los resultados de contaminación y otros factores fisicoquímicos del sedimento. La conclusión más convincente sería la de relacionar estadísticamente los resultados de toxicidad con concentraciones de contaminantes específicos o con determinados grupos de contaminantes. Según los resultados del análisis multivariante parece de especial interés la correlación con altos pesos para el Hg, los PCBs y el contenido en materia orgánica del sedimento identificada en el factor 4, que también explica parte de los efectos tóxicos registrados en el ensayo Microtox®. Si suponemos que estas tres variables normalmente registran valores altos en sedimentos de puertos y áreas urbanas, principalmente relacionados con el vertido de residuos sin depurar, el Microtox® parece recomendable para la evaluación inicial de la toxicidad de sedimentos provenientes de este tipo de zonas.

La batería de efectos tóxicos medidos no se relaciona con las mismas especies de contaminantes. Además la toxicidad

and is related to the toxic effects measured by half of the laboratories that reported results for the Microtox® bioassay. On the other hand, factor 5 groups the effect of the Ni concentration and the toxic effects registered by the sea urchin embryos. Some contribution to the decrease in luminescence was also identified.

Even though the Phase II results are similar to those reported in Phase I, the reproducibility of the toxic responses in Phase II seems more conservative, especially for the new Microtox® BSPT protocol. The multivariate analysis identified a strong relationship between Hg, PCBs and the organic matter content of the sediment in factor 4 that also explains part of the toxic effects. On the other hand, the contribution of some metallic compounds to the toxic response registered in the sea urchin embryo assay, such as those of As, Cr, Pb and Zn, appeared in both phases, though Hg and Ni were also identified in Phase II.

Discussion

The results of the interlaboratory comparison for the Microtox® and amphipod bioassays are satisfactory according to the development conditions and especially to the particular circumstances for each laboratory. The bioassay using clams did not produce satisfactory results because of the small number of laboratories that could be included in the study, though it is feasible for sediment toxicity assessment and further development is required for its use in relation to dredged material management. Moreover, the sensitivity of this organism is lower than that observed for the amphipods, the latter proving far more suitable for dredged material quality assessment. The interlaboratory study was repeated for the

Tabla 4. Peso de los tres factores principales para cada una de las 27 variables. Sólo aquellos pesos superiores a 0.25 están incluidos. Las medidas de toxicidad son A# = 100/EC50 SPT, B# = 100/EC50 BSPT, C# = % de larvas anormalmente desarrolladas; # son los códigos asignados previamente a cada laboratorio.

Table 4. Sorted rotated factor loadings of 27 variables in the five principal factors. Only loadings greater than 0.25 are included. Toxicity endpoints are A# = 100/EC50 SPT, B# = 100/EC50 BSPT, and C# = percent of abnormal sea urchin larvae; # indicates the code number assigned to each laboratory.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
% sand	–0.348	–0.900	–	–	–
% fine sediment	–	0.952	–	–	–
OM	0.601	0.428	–	0.592	–0.312
As	–	0.379	0.892	–	–
Cd	0.680	–0.458	0.405	–	–0.348
Cr	0.337	0.788	–0.321	–	0.331
Cu	–	–	0.969	–	–
Hg	–	0.538	–	0.795	–
Ni	–	–	0.388	0.292	0.874
Pb	0.621	–	0.772	–	–
Zn	–	–	0.971	–	–
Σ_7-PCBs	0.825	–	–0.329	0.402	–
Σ_{16}-PAHs	0.969	–	–	–	–
A1	0.970	–	–	–	–
A2	0.986	–	–	–	–
A3	0.879	–	–	0.395	–
A4	–	–	–	0.957	–
A5	0.679	–	0.420	0.429	0.408
A6	0.983	–	–	–	–
A7	0.353	0.271	–	0.709	0.519
B1	0.988	–	–	–	–
B2	0.984	–	–	–	–
B3	0.966	–	–	–	–
C1	–	0.801	0.338	–0.297	0.366
C2	–	0.605	–0.369	–	0.701
C3	0.473	0.337	–	–	0.784
C4	0.554	0.671	–0.263	–	0.403

para cada una de las especies y las medidas registradas no siguen las mismas tendencias en todos los casos (tabla 5) y estas diferencias se pueden también identificar mediante el análisis multivariante de los resultados. Se recomienda una batería de ensayos que incluya distintas medidas y distintos medios de exposición para proteger de modo efectivo todos los compartimentos ambientales. En este sentido y de acuerdo con los resultados de este ejercicio, una batería que incluya el ensayo Microtox®, el bioensayo con anfípodos desarrollado sobre la fase sólida y el ensayo de desarrollo larvario con embriones de erizo de mar sobre los lixiviados de los sedimentos, parece ser adecuada para la gestión de la toxicidad de sedimentos de dragado. Las distintas especies que se usaron han mostrado resultados satisfactorios si los criterios de

bioassays using sea urchin larvae and Microtox® since the results were not satisfactory. Some interfering factors may have critically influenced the results during Phase I and were thus avoided during Phase II, after which the interlaboratory variability decreased and was in the range of previously reported studies. The state of homogenization of the protocols to ensure the compatibility of results obtained by different technicians indicates that sediment bioassays are suitable tools, but if they are intended for use in a regulatory context, the staff should be trained and the validity of the test results should be certified through periodic exercises to evaluate inter- and intra-laboratory variability.

It is difficult to interpret toxicity tests and to relate the results to the mixture of potential environmental contaminants

Tabla 5. Caracterización de los materiales de dragado según la comparación de los resultados químicos con las guías de calidad de sedimento para la caracterización de material de dragado (CEDEX, 1994) y comparada con la caracterización de la toxicidad mediante ensayos de toxicidad en el laboratorio.
Table 5. Dredged material characterization based on the comparison of the chemical results with the traditional sediment quality guidelines for dredged material characterization (CEDEX 1994) and compared with the toxicity characterization by laboratory toxicity tests.

Sample	Category	Chemicals of concern	Microtox® SPT	Microtox® BSPT	Bioassay using amphipods	Bioassay using juvenile bivalves	Bioassay using sea urchin embryos
A	I	–	Not toxic	–	Not toxic	Not toxic	Toxic
B	III	PCBs	Toxic	–	Toxic	Not toxic	Toxic
C	III	As and Cu	Toxic	–	Toxic	Toxic	Toxic
D	III	PCBs and high PAHs	Toxic	–	Toxic	Not toxic	Not toxic
E	III	Cu, Hg and PCBs	Toxic	–	Toxic	Moderately toxic	Moderately toxic
F	III	Cd, Cu, Hg and PCBs	Toxic	–	Toxic	Moderately toxic	Moderately toxic
A’	I	–	Not toxic	Not toxic	–	–	Not toxic
B’	III	PCBs	Toxic	Toxic	–	–	Not toxic
C’	III	As, Cd, Cu and Ni	Toxic	Toxic	–	–	Toxic
D’	III	As, Cd and Cu	Toxic	Toxic	–	–	Toxic
E’	III	PCBs high PAHs	Toxic	Toxic	–	–	Toxic
F’	III	PCBs and high PAHs	Toxic	Toxic	–	–	Toxic

toxicidad se refieren a los resultados de un control de toxicidad negativo y si se utilizan técnicas estadísticas adecuadas para identificar diferencias en las respuestas. La selección de las especies para cada caso de estudio debe hacerse de acuerdo a las circunstancias particulares, pero siempre que se pueda asegurar que los resultados pueden ser utilizados para la toma de decisiones. Las agencias españolas a utilizar los resultados de los ensayos deberían ser las encargadas de decidir los criterios de aceptabilidad.

Finalmente, y de acuerdo con los resultados obtenidos para contaminantes bioacumulables no asociados a ninguno de los efectos agudos medidos, debería recomendarse para el futuro ensayos nuevos y específicos para evaluar la bioacumulación y sus efectos potenciales en el ambiente y en la salud humana, especialmente para contaminantes especialmente dañinos como por ejemplo las dioxinas o los PAHs. En resumen, esta es la primera iniciativa española para la evaluación interlaboratorio de ensayos de toxicidad con muestras de dragados portuarios. En cualquier caso los resultados no son definitivos ya que los ensayos deberían someterse a ejercicios periódicos que ayudasen a la mejora continua de los protocolos y a asegurar que los resultados de los laboratorios cumplen ciertos requisitos de calidad.

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and natural physicochemical factors. The most convincing results would be those that are based on demonstrating a significant relationship between multiple measures of toxicity and specific contaminants or groups of contaminants. The strong relationship between Hg, PCBs and the organic matter identified by the multivariate analysis in factor 4, which also explains part of the toxic effects registered by the Microtox®, is of special concern. Since high values of these three variables are expected in commercial ports and urban areas, principally due to untreated urban waste and the routine activities of ports and waterways, the Microtox® bioassay seems suitable for screening sediment toxicity in these types of zones.

The whole battery of toxic effects measured was not related to the same set of contaminants. Moreover, the toxicity measured for each test species and the endpoints do not follow the same patterns in all cases (table 5); these differences are also identified by means of multivariate statistical techniques. A battery of tests that includes different endpoints through different exposure routes and modes of action is recommended to effectively protect the environment. A battery that includes the Microtox® screening test, the bioassay using amphipods developed on the whole sediment and the bioassay using sea urchin larvae developed on the elutriate is suitable for this particular objective based on the results obtained in this study. The different species reported satisfactory results if the toxicity criteria are related to a control or reference sediment, using proper statistical analyses to elucidate significant differences. Test species for a particular case study should be selected according to each particular circumstance but always ensuring that the results can be used in a regulatory context. The Spanish agencies that will be the final users of the test results should decide the acceptability criteria for these techniques.

Based on our results concerning contaminants not clearly associated with the acute endpoints and possibly bioaccumulated early, new specific tests should be incorporated to

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properly address environmental and human health issues. In summary, this is the first initiative in Spain to include bioassays for regulatory purposes; however, the outcome is not definitive and continuous improvements are recommended to ensure the good quality of results.

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Capítulo 5.

Evaluación de la bioacumulación potencial de contaminantes asociados a materiales de dragado

1. Biodisponibilidad, bioacumulación y biomagnificación

La mayoría de contaminantes del medio acuático se encuentran presentes de forma natural a bajas concentraciones. No obstante, un aporte antropogénico masivo como puede ser el vertido de grandes volúmenes de materiales de dragado, puede aumentar de forma considerable las concentraciones de estos compuestos en el medio receptor. Así, estos contaminantes presentes a bajas concentraciones en agua, sedimentos y tejidos de organismos vivos pueden ser acumulados en mayor o menor medida si se dan las condiciones necesarias. La concentración y la forma de un contaminante en el medio ambiente determinan el grado de afinidad de este compuesto por los tejidos de los organismos y los efectos biológicos producidos en éste y en sus consumidores, incluido el hombre (Neff, 2002), o lo que es lo mismo, va a determinar su biodisponibilidad.

Se define biodisponibilidad como la afinidad de un contaminante para ser absorbido o adsorbido por un organismo vivo mediante procesos biológicos activos o procesos pasivos de tipo físico o químico. Todos los organismos vivos están separados del medio en que viven por membranas celulares o epitelios compuestos por una doble membrana lipídica con moléculas proteicas y canales cargados electroquímicamente. Por lo tanto, aquellos compuestos que sean biodisponibles serán introducidos a través de las membranas biológicas de forma pasiva o serán transportados mediante gradientes físico-químicos o sistemas enzimáticos y pueden ser así acumulados en los tejidos de los organismos.

Por la distinta naturaleza y mecanismos que determinan su comportamiento ambiental, se ha distinguido generalmente entre contaminantes de tipo orgánico e inorgánico, entre los que cabe destacar a los metales. Todos los seres vivos acumulan cierta cantidad de algunos metales traza para satisfacer los requerimientos mínimos para su metabolismo, son los denominados metales esenciales. Las concentraciones en los tejidos de estos organismos marinos pueden ser reguladas dentro de un intervalo estrecho de concentraciones excepto cuando las concentraciones ambientales alcanzan valores casi letales. Muchos de estos elementos esenciales son incorporados a través de las membranas biológicas mediante bombas proteicas, aunque la biodisponibilidad de un metal en solución va a depender de su actividad, entendida como la proporción de metal que está en su forma más biodisponible. Generalmente los iones libres, y sus diferentes estados de hidratación, son las formas biológicamente más activas aunque los metales presentes en sedimentos estuáricos y marinos, procedentes de muy distintas fuentes, están presentes en varias formas químicas –lo que se denomina especiación-.

La especiación de un metal va a tener un gran efecto en la biodisponibilidad y toxicidad de este metal. La especiación de metales en sedimentos anóxicos está controlada por la concentración de sulfuros presentes. Mediante el proceso de sulfato reducción, la materia orgánica se oxida y se descompone dando lugar a la formación de sulfuros metálicos, compuestos estables e insolubles en la mayoría de los casos. Los procesos que controlan la especiación de metales en sedimentos superficiales son más complejos, ya que los compuestos metálicos se distribuyen entre un mayor número de fases del sedimento -sedimentos de grano fino, óxidos de hierro, aluminio y manganeso, minerales arcillosos, carbonatos y materia orgánica- y esta distribución va a estar influenciada por un gran número de factores que incluyen la capacidad de unión de cada fase, la fuerza de estas uniones, la presencia relativa de las distintas fases del sedimento, otros parámetros fisicoquímicos como el pH, Eh o la presencia de ligandos que pueden influir la especiación del metal en disolución, así como la concentración de otros iones metálicos y no metálicos que pueden competir con los lugares de unión disponibles. Una gran proporción de los metales presentes en los sedimentos marinos se encuentra en su forma detrítica no disponible, es lo que se denomina fracción residual y está constituida principalmente por minerales duros y arcillas. Generalmente la fracción de metal que está disponible aumenta con la contaminación antropogénica. Por ejemplo, estas proporciones varían entre el 0-15% en sedimentos de Spitzbergen, Noruega, o el 1-27% en sedimentos de la bahía de Fundy (Neff, 2002). La biodisponibilidad de los contaminantes de tipo orgánico no polares

también va a depender de la forma física y química en la que se encuentre en el medio. Estos compuestos van a estar también menos biodisponibles si se encuentran en forma de compuestos adsorbidos, u otras formas sólidas. En general las formas disueltas son las más disponibles, aunque como en el caso de los metales, su biodisponibilidad va a estar determinada por un gran número de factores.

Se define el proceso de bioacumulación como la incorporación y retención de un contaminante biodisponible a través de cualquier fuente externa, bien agua, comida, sustrato o aire. Por lo tanto se considera el resultado neto de la incorporación, distribución y eliminación de una sustancia en un organismo debido a las múltiples fuentes de exposición (ECETOC, 1996). Algunos compuestos muy solubles pueden ser incorporados a través de las membranas, pero son rápidamente excretados por difusión, transformación metabólica o transporte activo y por lo tanto las concentraciones en los tejidos se mantienen a niveles más o menos constantes independientemente de la concentración ambiental en el medio. Por el contrario, la exposición continua de un organismo a concentraciones más o menos constantes puede causar la incorporación de contaminantes en los tejidos hasta una determinada concentración más o menos estable. La concentración de equilibrio entre la entrada y salida se mide normalmente como el factor de bioacumulación (en sus siglas inglesas BAF), calculado como el cociente entre la concentración de un contaminante en el tejido y la concentración en los distintos compartimentos ambientales en equilibrio con el organismo. Los organismos marinos son capaces de bioacumular la mayoría de formas biodisponibles de contaminantes orgánicos y metales que forman parte de su alimento, es lo que se denomina bioacumulación a través de la cadena trófica. El proceso de biomagnificación se define generalmente como el proceso de bioacumulación a través de distintas rutas en los distintos niveles tróficos (Beek et al., 2000). Esto no significa que todos los compuestos químicos se biomagnifiquen, es decir que se encuentren a concentraciones más elevadas en organismos localizados en los niveles superiores de las redes tróficas, ya que muchos compuestos pueden sufrir procesos de metabolización y depuración. Existen dos condiciones para que se produzcan efectos de biomagnificación. En primer lugar el compuesto debe ser incorporado fácilmente en los procesos digestivos, después y una vez el compuesto está unido a los tejidos del consumidor, debe ser retenido. Si ambas condiciones se cumplen el compuesto químico permanece durante largo tiempo y, si el consumidor sigue alimentándose repetidamente de alimentos contaminados, puede llegar a acumular concentraciones superiores a las encontradas en su alimento. Según Suedel et al. (1994) los contaminantes DDT, DDE,

PCB, toxafeno, metilmercurio, mercurio total y arsénico tienen la capacidad de biomagnificarse en el medio acuático.

2. Bioacumulación de contaminantes procedentes de materiales de dragado

El vertido de grandes volúmenes de material de dragado contaminado puede suponer un aporte de contaminantes al medio. Si estos contaminantes son compuestos que pueden sufrir un proceso de bioacumulación o biomagnificación en el medio acuático, la caracterización de los materiales de dragado previa licencia para ser vertidos al mar debe incluir la evaluación de los efectos indirectos producidos en la red alimenticia local y en organismos localizados en niveles superiores de la cadena trófica (PIANC, 2006). El objetivo es prever un posible efecto sobre la salud humana. Las RGMMD (CEDEX, 1994) incluyen el desarrollo de ensayos biológicos para aquellos materiales con concentraciones del grupo A (Hg, Cd, Pb, Cu, Zn, Ni, Cr, ΣPCBs) superiores al 75% del NA2 o en caso de que los materiales presenten concentraciones elevadas de los contaminantes incluidos en el grupo B (As, otros PCBs, PAHs, aceite y grasa, y otros). Estos ensayos biológicos deberían incluir estudios de asimilación de sustancias nocivas aunque, como en el caso de otro tipo de ensayos biológicos, estas metodologías no se encontraban en un estado de desarrollo tal que permitiese su uso en un contexto regulador.

Hoy en día los efectos indirectos por bioacumulación de contaminantes presentes en materiales de dragado son considerados de forma paralela a otro tipo de efectos tóxicos directos durante los procesos de caracterización y gestión si los sedimentos presentan contaminantes potencialmente sujetos a procesos de bioacumulación y biomagnificación (PCBs, DDT, mercurio, etc.). Cuatro son las metodologías disponibles para la evaluación de este tipo de procesos en materiales de dragado (Peddicord et al., 1997):

1) Estudios de laboratorio que exponen los organismos al sedimento bajo condiciones controladas
2) Estudios de campo recogiendo especies de la zona considerada
3) Estudio de la transferencia a través de la red trófica
4) Modelos para predecir los procesos de bioacumulación.

Siguiendo un procedimiento escalonado, una evaluación preliminar de la contaminación de los sedimentos por comparación con algún tipo de guías químicas de calidad puede ser útil para estudiar el potencial/probabilidad de efectos adversos en

organismos que serían expuestos a esos sedimentos. En los últimos años se han establecido numerosas guías químicas de calidad de sedimento, aunque cabe mencionar que muchas de ellas representan simplemente condiciones de fondo o alguna medida de concentraciones ambientales que se consideran aceptables desde un punto de vista político y/o económico, o bien han sido concebidas para predecir la ausencia de efectos tóxicos directos (por ejemplo, las guías ERL-ERM). En resumen, pocas son las guías químicas que consideran este tipo de procesos en su desarrollo y que puedan ser utilizadas como evaluación inicial de efectos bioacumulativos y de biomagnificación. Además, el método rutinario para el análisis de la contaminación en sedimentos, que considera sólo concentraciones totales, se considera una aproximación conservativa en cuanto a la salud humana y el medio ambiente, aunque el riesgo puede sobreestimarse dando lugar a estudios más largos y costosos (Bradham et al., 2006). Debido al mayor coste de los ensayos de bioacumulación en el laboratorio, en los últimos años se han desarrollado métodos alternativos para la evaluación de la bioacumulación potencial, como por ejemplo el Potencial de Bioacumulación Termodinámico, en sus siglas inglesas TBP. Este método hace uso del principio de equilibrio de partición para compuestos químicos no polares con objeto de estimar la concentración que puede pasar de la fase de carbono orgánico del sedimento a la fase lipídica de un organismo cuando se alcanza el estado de equilibrio. Numéricamente, el TBP se calcula mediante la expresión

$$TBP=BSAF\ (Cs/foc)fl$$

donde:

TBP= concentración de contaminantes en las mismas unidades que Cs

BSAF= factor de acumulación biota-sedimento

Cs= concentración del contaminante orgánico no polar en el sedimento (expresado como peso seco)

foc= contenido en carbono orgánico total del sedimento expresado como fracción

fl= porcentaje de contenido lipídico en el organismo expresado como fracción del peso húmedo del organismo.

El valor de BSAF se estima empíricamente mediante ensayos de bioacumulación previos o bien mediante estudios de campo donde se disponen de datos de concentraciones en tejido y organismo así como del contenido lipídico del organismo y del contenido en carbono orgánico total del sedimento. Con estos datos el BSAF puede ser calculado como

$$BSAF = (Cb/fl)/ (Cs/foc)$$

donde:

Cb= concentración de contaminante en el organismo en un estado de equilibro

Aunque este tipo de método parece estimar el potencial de bioacumulación de compuestos orgánicos apolares (Clarke y McFarland, 2002) también ha sido aplicado a metales por su gran simplicidad, a pesar de las diferencias en las propiedades físicas, químicas y toxicológicas de estos compuestos orgánicos e inorgánicos (McGeer et al., 2003).

Recientemente se han publicado un gran número de estudios que cuestionan el uso de este tipo de modelos para describir la relación entre bioacumulación y sus efectos en los organismos por la presencia de sustancias de tipo inorgánico como los metales (Franke, 1996; Chapman et al., 1999). En general la bioacumulación de un compuesto no tiene por qué causar efectos adversos en el organismo que sufre esta acumulación. Por ejemplo, en el caso de nutrientes esenciales, cierta cantidad es necesaria para cubrir las funciones fisiológicas normales del organismo. En cualquier caso, parece lógico que un simple cociente no sea suficiente para integrar los procesos de incorporación, secuestro interno, almacenamiento, eliminación activa, o la esencialidad o toxicidad potencial, es decir, para determinar la compleja dinámica de un determinado metal (McGeer et al., 2003).

Un segundo método para estudiar el potencial de bioacumulación de los contaminantes presentes en materiales de dragado incluye el desarrollo de ensayos en el laboratorio para determinar el movimiento de los contaminantes desde el sedimento a los tejidos de los organismos expuestos. Los ensayos de bioacumulación se desarrollan bajo condiciones controladas con organismos que viven en contacto con el sedimento, cuyos tejidos son analizados tras finalizar el periodo de exposición. Las especies de ensayo deben ofrecer un nivel de tolerancia mínimo a los contaminantes presentes y, además, proporcionar una cantidad suficiente de tejido para ser analizada mediante la tecnología disponible. Cabe mencionar que los ensayos de bioacumulación ofrecen una medida de la exposición y no un efecto, por lo tanto los resultados obtenidos de este tipo de ensayos no pueden ser interpretados de forma similar a la utilizada en otros ensayos biológicos de toxicidad. En general, el estudio de la bioacumulación en un material de referencia en paralelo a los materiales de dragado permite identificar los sedimentos que potencialmente pueden provocar procesos de bioacumulación en organismos bentónicos, si bien el estudio

de la bioacumulación en procesos de gestión de material de dragado necesita contrastar cinco hipótesis que resumen de forma concisa la información necesaria para la toma de decisiones (Peddicord et al., 1997):

1) ¿Para cuántos contaminantes la bioacumulación en los materiales de dragado excede a la bioacumulación en el material de referencia?
2) ¿En qué magnitud excede la bioacumulación en los materiales de dragado a la bioacumulación en el material de referencia?
3) ¿Cuál es la importancia toxicológica de los contaminantes cuya bioacumulación en el material de dragado excede a la producida por lo material de referencia?
4) ¿Cuál es la tendencia del contaminante a sufrir procesos de biomagnificación dentro de la red alimenticia acuática?
5) ¿Exceden los contaminantes identificados previamente también las concentraciones de estos compuestos en organismos que viven en los alrededores de la zona de vertido?

Para dar respuesta a estas preguntas se han puesto en marcha distintas iniciativas, principalmente desde el cuerpo de ingenieros estadounidense (en sus siglas inglesas US ACE), para desarrollar una base de datos de BSAF y contenido lipídico disponible en internet[1] y se está recopilando toda la información disponible donde se relacionan efectos con concentraciones de contaminantes en tejido[2].

En este capítulo se resumen los resultados de distintos ensayos de laboratorio para el estudio de posibles procesos de bioacumulación en organismos bentónicos por exposición a materiales de dragado. Siguiendo las recomendaciones y metodologías en uso para este tipo de estudios se han utilizado poliquetos de la especie *Arenicola marina,* que ofrecen numerosas ventajas como especie de ensayo respecto a otras especies bentónicas (ver capítulo 3 de esta tesis) y no registran una disminución considerable de la supervivencia por exposición a este tipo de materiales durante los ensayos en el laboratorio. Por razones muy similares, se consideró también el estudio de la bioacumulación con almejas de la especie comercial *Ruditapes philippinarum*, siguiendo el protocolo habitual a 28 días de exposición a los sedimentos en bruto. Las concentraciones en tejidos tras exposición a los materiales de

[1] http://el.erdc.usace.army.mil/bsaf/bsaf.html
[2] http://el.erdc.usace.army.mil/ered/index.html

dragado han sido evaluadas en relación a las concentraciones en tejidos tras exposición a un material de referencia y se han estudiado los resultados en relación al cuadro de gestión recomendado para materiales de dragado. Los resultados para los compuestos de tipo metálico en *A.marina* (excepto Hg), resumidos en el trabajo XIII, muestra la posible acumulación de estos contaminantes tras la exposición a materiales de dragado. Las concentraciones medidas en los organismos se encontraban dentro del rango de concentraciones registradas en estudios de campo y laboratorio para esta misma especie aunque los sedimentos considerados en este estudio obtuvieron concentraciones de algunos contaminantes muy superiores a los obtenidos en estos estudios.

Los resultados muestran una acumulación evidente en los organismos expuestos a las mayores concentraciones de metales (concentraciones que superan los NNAA2 y que, en algunos casos, clasificaban las muestras como materiales de Categoría IIIb), para los cuales también se registró una alta mortalidad. En este sentido se puede decir que las concentraciones totales en sedimento pueden predecir una bioacumulación potencial en este organismo bentónico, aunque sólo cuando las concentraciones acumuladas por los poliquetos son lo suficientemente elevadas como para causar efectos tóxicos significativos. A concentraciones intermedias la variabilidad es mayor, es decir, cuando las concentraciones de metales en el organismo no superan el umbral de toxicidad es más difícil establecer una relación causa efecto y utilizar la concentración total en sedimento para establecer si existe algún riesgo por el vertido de los materiales de dragado, posiblemente por el gran número de factores abióticos que determinan la biodisponibilidad final de estos metales.

Los metales no esenciales Cd, Cr, Ni y Pb se bioacumulan más en *A.marina* que otros metales no esenciales como el As, Cu o Zn. Además, los pares de datos de concentraciones en sedimento y organismos para estos metales no esenciales se ajustan mejor a los modelos considerados. Cabe mencionar que, teniendo en cuenta las diferencias entre los sedimentos de este estudio, los resultados tras la corrección por el contenido en materia orgánica del sedimento, permite reducir la variabilidad de los resultados a concentraciones intermedias por lo que la materia orgánica del sedimento va a ser un factor determinante cuando se estudia la biodisponibilidad de metales unidos a materiales de dragado.

Los resultados para este tipo de compuestos en almejas de la especie *R.philippinarum*, resumidos en el trabajo XV, muestran la bioacumulación de todos los compuestos considerados excepto el As, que parece no ser acumulado por este organismo bajo las condiciones de ensayo. Las concentraciones medidas en este organismo tras la exposición a

los materiales de dragado se correlacionaron mejor que los resultados para el poliqueto *A.marina* con las concentraciones en sedimento, con una menor variabilidad de los resultados dentro de los rangos para las muestras de este estudio. El contenido en materia orgánica del sedimento parece ser un factor determinante en la biodisponibilidad y bioacumulación de contaminantes en organismos bentónicos para algunas muestras. La corrección de las concentraciones en sedimento para el contenido en materia orgánica parece ser un buen modelo para el estudio de la bioacumulación potencial de Cu en los dos organismos estudiados, pero también de Pb y Zn en poliquetos.

El estudio de los cocientes de bioacumulación -BAF y BSAF cuando se tiene en cuenta el contenido en materia orgánica del sedimento-, que es uno de los modelos utilizados en estudios de bioacumulación y biodisponibilidad, identificó unos valores muy variables para cada sedimento y para los metales estudiados. El uso de un valor estándar, similar al utilizado para contaminantes de tipo orgánico, debería ser utilizado como metodología de "screening" inicial tal y como recomiendan distintas agencias ambientales. El uso de valores muy restrictivos puede ser suficiente para considerar que no existe riesgo en el vertido de los materiales de dragado, al igual que unas concentraciones extremadamente elevadas en sedimento puede ser una evidencia clara de toxicidad. El uso de modelos que tengan en cuenta un mayor número de factores bióticos y abióticos ayudará en estudios de bioacumulación, aunque en estos momentos parece recomendable acompañar este tipo de estudios con ensayos de laboratorio, especialmente si se tienen en cuenta los costes asociados a la gestión de materiales de dragado. En este tipo de ensayos se recomienda el uso de estaciones control para establecer las concentraciones en organismos bajo unas condiciones de referencia y que pueden ser utilizados como criterio de calidad biológico similar a los SQGs y NNAA químicos y poder establecer así la importancia ecotoxicológica de los metales en los organismos bentónicos y dentro de la cadena trófica en caso de ser contaminantes que sufren biomagnificación.

En este grupo de contaminantes destacan los compuestos orgánicos del Hg, los bifenilos policlorados e hidrocarburos aromáticos –PCBs y PAHs respectivamente-. Según los resultados de este estudio para estos compuestos (trabajo XIV) los individuos de esta especie muestran cierta tendencia a acumular mayores concentraciones de PCBs a concentraciones mayores en sedimento, cosa que también sucede para el Hg y para los compuestos orgánicos del Hg. La biodisponibilidad de estos compuestos parece estar determinada por el contenido en materia orgánica del sedimento, aunque el uso del modelo BAF/BSAF puede ofrecer resultados controvertidos de acuerdo a la gran variabilidad en

los valores obtenidos para estos cocientes de bioacumulación. El uso del valor estándar establecido por la agencia ambiental americana igual a 4 parece ser una metodología protectiva en relación a los valores obtenidos en este estudio, que están un orden de magnitud por debajo. Esto también sucede en relación a los compuestos de tipo PAHs aunque la bioacumulación de este tipo de compuestos parece mucho más variable y no sigue el gradiente de concentraciones en sedimento. Para estos compuestos la corrección para el contenido en materia orgánica del sedimento no parece ser el factor responsable de esta variabilidad y, por lo tanto, parecen verse afectados por un mayor número de procesos físico-químicos y/o biológicos que dificulta la relación causa-efecto entre concentraciones en sedimento y concentraciones en organismos. Las bajas concentraciones de los resultados para organismos expuestos a un sedimento control o de referencia no permiten evaluar la medida de la bioacumulación en los sedimentos de dragado, sin embargo los resultados parecen indicar que este organismo es adecuado para evaluar la bioacumulación de contaminantes presentes en materiales de dragado bajo condiciones de laboratorio.

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Experimental studies on bioaccumulation of metals from dredged sediments to the lugworm (*Arenicola marina*, Linnaeus, 1758)[1]

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Abstract

This paper presents the results of metal bioaccumulation from dredged sediments to the lugworm *Arenicola marina* upon laboratory exposure. The sediment concentration/lugworm concentration pairs indicate a linear positive relationship for all the metals except for the metalloid As, for which no relationship between concentrations in sediments and in lugworms could be established. The non-essential metals Cd, Cr, Ni and Pb presented a higher bioaccumulation in lugworms than the essential metals As, Cu and Zn, with higher slopes and also higher correlation coefficients (0.48-0.73 for non-essential and 0.01-0.25 for the essential metals). Even though it is difficult to identify an straightforward relationship between the concentrations of metals in sediments and the concentrations in lugworms upon exposure to dredged materials in the laboratory, and despite the differences in the grade and the sources of contamination in the sediment samples studied, the methodology deployed was useful to study the bioaccumulation potential of metals from dredged sediments to this benthic invertebrate. This type of methodology may be especially recommended to assess the risks of bioaccumulation from dredged materials with intermediate levels of contamination, where the results evidenced a higher variability in the bioavailability of metals than at extreme contamination levels.

Keywords: sediment quality assessment, trace metals, polychaetes, BAF, BSAF.

[1] Environmental Pollution (Enviado)

1. Introduction

Contaminants enter aquatic systems through a variety of point and nonpoint sources and in time become associated with sediments. Because both anthropogenic and naturally occurring trace elements can become biologically available by changing sediment-contaminant associations (Peddicord et al., 1997), sediments are the subject of environmental concerns if they are excavated and remobilized. Several frameworks designed to guide dredged material management recommend to assess the bioaccumulation potential in relation to biomagnication through food chain together with acute toxicity assessments (PIANC, 2006; US EPA, 1991). Current routine practices for investigating the nature and extent of contamination involve determining total content in sediments as an estimation of exposure but for a chemical to bioaccumulate/biomagnify it must be available for bioaccumulation and, once it has entered the food chain it must be subject to biomagnification in top predators (three chains). Thus, as for acute toxicity, different physico-chemical properties of contaminants and sediments can affect bioavailability and specific issues may be encountered locally and regionally which determine that chemical analyses alone are not good predictors of bioaccumulation in a biological system. While this "chemical" approach is conservative in terms of being overprotective of human health and the environment, the true risks may be overestimated, resulting in lengthly and costly management decisions (Bradham et al., 2006).

In the case of dredged materials the potential to move into the food chain and produce effects in organisms above and beyond the borders of a disposal site begins with assessing bioaccumulation potential (PIANC, 2006). As described by Peddicord et al. (1997) four general approaches are used in risk assessment with respect to bioaccumulation: 1) a laboratory approach exposing organisms to sediment under controlled conditions, 2) a field approach collecting species from a study area, 3) assessment of food web transfer and 4) models to predict bioaccumulation processes. Much research has been performed on single, spiked contaminants in specific soils and sediments to predict bioaccumulation processes through conservative models and further assess food web transfer. Although this theoretical approach seems to be a powerful method to screen for "bioaccumulation hot spots", such evaluations have little environmental relevance since sediments consist of a mixture of weathered chemicals in a heterogeneous soil matrix (Chapman and Loehr, 2004). Bioaccumulation potential from

dredged sediments can be also assessed through a field sampling campaign to collect organisms living on the sediments to be dredged, but this is sometimes difficult due to the high disturbance of the bottom surface in ports and navigational channels. In this context laboratory bioassays that allow for direct measures of tissue concentrations in specific organisms representative of the biota under risk are recommended (PIANC, 2006; Chapman and Anderson, 2005), even though this approach needs of sound knowledge on the biology of the test organism and the behaviour of contaminants.

This paper presents the results of laboratory tests for the assessment of metal bioaccumulation from dredged sediments in the marine benthic organism *Arenicola marina* (Polychaeta). The lugworm *Arenicola marina* is an infaunal invertebrate living in intimate contact with the sediment, where it burrows and feeds. This organism is in direct contact with sediment-associated contaminants, being a surface deposit feeder feeding on the organic fraction of the just-sedimented detritus of the ingested sediment. This species is commonly found in ports and harbours, where they can account for a high proportion of the total biomass. It is an important prey item and can contribute to the transport of contaminants to higher levels in the food chain. In addition it has been widely studied and used in different toxicity and bioaccumulation studies (i.e. Casado-Martínez et al., accepted; Bernds et al., 1998; Kaag et al., 1998). The results of bioaccumulation from dredged sediments in lugworms following the standard 10 day operational procedure are presented. A shorter exposure period than the current 28 days, equivalent to the acute toxicity test protocol has been considered according to previous studies with this species (Bernds et al., 1998; Christensen et al., 2002; Geiszinger et al., 2002). The variability in metal accumulation within and between ports is further discussed.

2. Material and methods

2.1. Test sediments and chemical characterisation

Sediments from four ports were included in this study: Barcelona and Cádiz (named B# and CA# respectively) are both fishing and shipping ports near major centres of population; Cartagena and Huelva are in a region hosting important mining districts, chemical and metalurgical factories, power plants, oil refineries, and a celulose factory in Huelva. In each port three to four sampling stations were selected, all representative of dredging operations, to a total number of thirteen. In addition, one sampling station was

selected as control sediment based on historical data (Riba et al., 2003). Sediments were collected with a 0.025 m^2 Van Veen grab from approximately the top 20 cm, deployed untill enough sediment was obtained at each location. The pooled samples were homogenised and the wet sediments were stored in clean polyethylene bottles at 4ºC and darkness prior to tests. Analytical methods were described in detailed elsewhere (Casado-Martínez et al., 2006). For the sediment characterisation a subsample was dried at 40ºC for 24 hours prior to analyses. Grain size distribution followed UNE 103 101 and total organic matter content (TOM) was estimated by loss of ignition at 550ºC followed by gravimetric determination. Metals were determined in microwave acid-digested samples in Teflon vessels. The concentrations of Cd, Pb, Cu, Zn and Cr were determined using flame or furnace atomic absorption spectrometry, depending on the metal content. For As the hydride generation technique was used followed by quantification using atomic absorption spectrometry. All the analytical procedures were checked with reference materials (MESS-1) and obtained concentrations did not differ significantly ($p<0.05$) from certified values.

2.2. Test organisms, experimental set-up and tissue measurements

Individuals of *Arenicola marina* (Linnaeus, 1758) (lugworms) were collected in the Cantabric coast (north of Spain) by hand digging and were shipped refrigerated to the laboratory in the University of Cádiz in clean seawater. Once in the laboratory the organisms were immediately transferred into 20 L holding tanks with a 5 cm clean sediment layer and clean seawater for acclimatization to test conditions (temperature and salinity) for at least 15 days prior to tests. Before being used, organisms were transferred to 20 L holding tanks with clean seawater for at least 24 hours for depuration.

The bioassay followed protocol by Thain and Bifield (2001) and has been described in detail elsewhere (Casado-Martínez et al., accepted). Sediments (2 L) were loaded into 12 L aquaria providing a layer of sediment 5 cm deep. Approximately after 12 hours clean sea water (6 L) was added to each container and then exposure chambers were left to settle for 12 hours before aeration was started. After 12 hours of aeration five lugworms were placed in three replicate containers. No extra food was added to the test organisms and overlying water was renewed every three days or if any mortality was registered. After 10 d, surviving worms were placed in clean seawater to depurate for 24 h, then blotted dry and frozen at −80°C for later tissue analysis. A negative control consisting of clean sandy sediment from Cádiz was used together with lugworms home sediment. For the analysis of metal content

in lugworms the liophilized organisms were pooled and tissues were extracted with H_2O_2 and HNO_3 at 95 ºC for 45 minutes. After digestion, the solution was brought to 10 ml volume with deionised water. Each digestion included at least one blank treatment and one standard reference material (DORM-1 or TORT-2). Analytical deteminations were performed by inductively coupled plasma mass spectrometry as described by (Martin-Diaz et al., 2006).

2.3. Data treatment and analysis

To have a measure of the accumulation of a metal in tissues the BioAccumulation Factors (BAF) were calculated individually for each metal using tissue residues attained by *A.marina* at day 10 and the concentration in sediment as follows:

$$BAF = M_{OS} / M_S$$

where $[M_{OS}]$ is the mean concentration of the metal (M) in the organism (O) exposed to the dredged material (S) and $[M_S]$ is the concentration of the metal (M) in the dredged material (S). In addition the Biota-Sediment Accumulation Factor (BSAF) was also calculated by normalizing the value for the organic matter content of the sediment as follows:

$$BSAF = M_{OS} / (M_S / f_{oc})$$

where $[f_{oc}]$ is the sediment organic content. To evaluate the magnitude of bioaccumulation of a metal (M) from the dredged materials as compared to control-sediments, BioAccumulation Ratios (BARs) were calculated as follows:

$$BAR = M_{OS} / M_{OC}$$

where $[M_{OC}]$ the corresponding mean concentration of the metal (M) in the organism (O) exposed to control-sediment (C). For comparative purposes sediment concentration ratios (SCRs) were calculated as follows:

$$SCR = M_S / M_C$$

where $[M_C]$ is the concentration of the metal M in the control-sediment (C).

3. Results and discussion

3.1. Grain size distribution and organic matter content

The results of the sediment physico-chemical characterisation are presented in Table 1 together with the limit values (namely Action Levels) used in Spain for dredged material characterisation and management. Briefly, these two limit values determine three different categories, which in turn determine different assessment and management needs. The control sediment was a typical sand with the lowest TOM content while the properties of the tested sediments differed within and between ports. Grain size and TOM varied a great deal inside ports and, even if the number of stations is insuficient to draw spatial variations and gradients within ports, organic matter seems to be preferentially accumulated in fine sediments deposited in inner parts (stations CA4, B4, C1 and H2). This resulted in a significant correlation between the TOM content and the proportion of fines in the sediments ($p<0.05$; Table 2).

3.2. Metals in sediments

The control sediment contained the lowest metal concentrations, far below the rest of sediments and the corresponding limit values for dredged material management (Table 1). The sediments from Cádiz reported the lowest metallic load according to the lower influence of industrial activities in this area although Cd concentrations were intermediate on dredged muds (CA3 and CA4). The sediments from the inner fishing harbour (CA2) presented the highest contamination with the Cu and Pb concentrations exceeding the lower Action Levels (AL1). This enrichment was not related to TOM or grain size as sediments from the stations CA3 and CA4 presented higher values on these variables due to urban wastes but lower metal concentrations than the sediments near the fishing harbour (CA2).

Table 1
Conventional parameters and metal concentrations in the sediments studied (Port of Cádiz, CA#; Port of Huelva, H#; Port of Barcelona, B#; Port of Cartagena, C#). The limit values used in Spain for dredged material management are included (AL1 and AL2; see text for further details). All concentrations expressed in mg/kg in dry weight basis.

Sample	% fines	TOC	As	Cd	Cr	Cu	Ni	Pb	Zn
CA1	0.04	1.07	3.42	0.92	0.1	6.98	0.06	2.28	21.27
CA2	59.53	13.75	30.77	1.32	14.94	202.8	20.14	86.9	378.25
CA3	81.90	20.30	16.61	1.23	8.43	46.76	16.9	17.61	135.5
CA4	99.59	24.33	7.81	1.25	14.22	32.07	21.25	5.14	65.67
H2	90.21	10.64	531	2.5	24.1	1497	7.1	384.7	1857
H3	43.95	6.30	273	1.32	8.13	772	129	217.6	1176
B1	33.86	3.06	17.39	0.93	105.2	74.88	18.87	86.66	253.8
B3	53.98	4.81	18.56	0.62	59.53	102.1	22.24	91.9	219.7
B4	58.70	17.56	28.99	2.88	93.86	601.1	32.3	455.3	1165
C1	57.81	10.54	101.5	98.49	66.64	665.9	29.04	1397	8661
C2	41.19	9.12	64.71	17.47	45.61	313.4	15.33	748.3	1885
C3	31.87	7.19	88	31.88	57.57	453.3	19.32	1397	3310
C4	49.10	9.87	62.55	6.79	29.48	171.1	19.32	486.7	900.8
AL1	-	-	80	1.0	200	100	100	120	500
AL2	-	-	200	5.0	1000	400	400	600	3000

Table 2
Correlation corefficients between the concentrations of metals and the variables related to the sediment properties. Significant correlations at $p<0.05$ are marked with an asterisk.

	As	Cd	Cr	Cu	Ni	Pb	Zn	% fines
As	1.00							
Cd	0.55	1.00						
Cr	0.67*	0.59	1.00					
Cu	0.92*	0.58	0.84*	1.00				
Ni	0.48	0.40	0.93*	0.64	1.00			
Pb	0.83*	0.80*	0.82*	0.90*	0.60	1.00		
Zn	0.85*	0.85*	0.82*	0.92*	0.62	0.98*	1.00	
% fines	0.27	-0.22	0.45	0.28	0.61	-0.03	0.07	1.00
TOM	-0.16	-0.33	0.38	-0.01	0.62	-0.21	-0.16	0.84*

The area of Huelva presented As and Cu concentrations failing the corresponding high limit value, which determine the classification of these sediments into the highest priority category. In addition these sediments presented intermediate concentrations of Cd, Pb and Zn. In the port of Cartagena the sediments were highly contaminated with Cd, Cu, Hg, Pb and Zn, with concentrations exceeding the corresponding limit values for open water disposal (AL2; Table 1). The highest concentrations of these metals were present at stations C1>C3 on the eastern bay and C2>C4 on the western bay. The port of Barcelona do not stand such determining sources of contamination and concentrations of metals varied with the distance to the inner harbors. The category in which each sediments fell decreased as the concentrations of trace metals decreased from the inner station B4, that fell into the highest priority category, and going seaward as B2 is category II and B1 is category I.

Despite the correlation analysis identified no significant correlation between the metal concentrations in sediments and the variables related to the sediment properties (Table 2), the results indicate that metals are principally associated to the fine sediment particles, usually rich in organic matter content. This is especially evident in the port of Huelva, located in the Tinto and Odiel estuary, and in Barcelona, where sampling stations followed a transect seaward along the port.

3.3. Mortality of lugworms exposed to contaminated sediments

The sediments from Cádiz and Barcelona caused no significant increase in lugworm mortality (at $p<0.05$), except CA4 that resulted in a mean mortality higher than 20%. The sediments from Huelva showed mortalities between 25% (H2) and 40% (H3) while the sediments from Cartagena presented very variable mortality results. The sediments from the eastern part of Cartagena were highly toxic and mortality was 67 and 80% for C3 and C1 respectively, while sediments from the western bay (C2 and C4) did not decrease survival in lugworms despite the high sediment contamination. Lugworm mortality was significantly correlated with the Cd, Pb and Zn concentrations in sediments due to the high toxicity presented by the most contaminated sediments from Cartagena ($p<0.05$; Casado-Martínez et al., accepted). The concentrations of As, Cu, Cr and Ni presented positive correlations with the lugworm mortality but not significant ($p<0.05$), while the variables that account for the sediment characteristics presented negative correlation coefficients, which indicate that the sediment properties do not account for the toxic effects measured.

3.4. Metal concentrations in lugworms exposed to contaminated sediments

The concentrations of metals in lugworms after 10 days of exposure to dredged sediments is presented in Table 3. Lugworms exposed to the control and reference sediments exhibited similar tissue concentrations, usually in the lowest range for all sediments and for all metals. Slight differences were reported for Cr, Cu and Pb, which were higher in control-exposed organisms than in reference sediment, and As, Ni and Zn, that were higher in the reference sediments than in the control sediment. Our results were always in the range of previous concentrations reported in lugworms except Cd, Pb and Zn that were accumulated to a higher extent in our study according to the higher metal concentrations in our sediments than in previous field and laboratory simulations (Table 4).

Table 3
Concentration of metals in organisms expressed as mg/kg on dry weight basis. CA# correspond to the Port of Cádiz, H# Port of Huelva, B# Port of Barcelona and C# Port of Cartagena. CA1 is the control sediment and Ref presents the results after exposure to the sediments where the lugworms were obtained.

	As	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
CA1	36.64	0.17	1.00	12.28	2.45	1.03	38.01
Ref	39.25	0.10	0.96	9.08	3.57	0.78	44.01
CA2	24.41	0.14	0.89	6.48	2.53	0.69	27.69
CA3	46.82	0.08	0.85	10.79	5.79	0.77	43.56
CA4	54.00	0.28	0.76	11.29	6.94	1.20	57.84
H2	55.01	0.10	1.13	16.60	3.20	2.09	39.83
H3	25.38	0.07	0.65	16.57	2.84	1.04	31.15
B1	55.53	0.10	2.61	13.12	6.44	1.06	55.99
B3	45.48	0.14	1.63	16.83	4.71	1.13	59.22
B4	32.29	0.16	1.52	15.48	7.28	1.22	55.03
C1	68.54	6.57	3.27	44.29	6.23	29.10	252.71
C2	38.12	0.29	0.59	10.09	2.94	2.14	64.91
C3	66.82	1.58	2.70	33.19	5.74	20.54	158.4
C4	29.38	0.15	0.69	11.35	2.85	3.24	53.48

Table 4
Ranges of concentrations of metals in lugworms and other field-collected polychaetes reported in the literature and in the present study (expressed in mg/kg).

	Wright and Mason (1999)[a]	**Neff (2002)[b]**	**Our data**
As	-	5.0-2740	24.41-68.54
Cd	0.48-1.21	0.12-45.0	0.10-6.57
Cr	-	1.1-65.5	0.65-3.27
Cu	14.3-41.7	6.5-106	6.48-44.29
Ni	4.12-101	-	2.45-7.28
Pb	1.27-1.89	0.89-82.1	0.69-29.10
Zn	121-170	92-297	31.15-252.71

[a]Field collected polychaetes in estuaries from eastern England;
[b]Data summarized in Neff (2002) from different studies.

3.5. Relationship between concentrations in sediment and in lugworms

A preliminary study of all sediment concentration/lugworm concentration pairs indicate a linear positive relationship for all metals except for the metalloid As that showed no relationship between concentrations in sediments and in lugworms (slope 0.0158, R^2=0.0029; Table 5, Fig. 1). The non-essential metals (Cd, Cr, Ni, Pb) presented a higher bioaccumulation in lugworms than the essential metals (As, Cu, Zn) as the slopes are higher (0.48-0.73 for non-essential and 0.01-0.25 for the essential metals). In addition the correlations were better for these non-essential metals than for the essential ones. An slight positive relationship was determined for Cr, Cu and Ni while the results for Cd, Pb and Zn were determined by the high concentrations in sediments and in lugworms after exposure to the most contaminated sediments C1 and C3, which presented the highest mortalities but also the highest concentrations of all metals in lugworms after the 10 days of exposure.

Table 5
Descriptors of the fitted line for the results found in this study when all concentrations in sediments and in lugworms are considered (Fig. 1).

Trace element	**Slope**	**R^2**
As	0.02	0.00
Cd	0.73	0.75
Cr	0.48	0.51
Cu	0.20	0.22
Ni	0.53	0.26
Pb	0.49	0.47
Zn	0.25	0.32

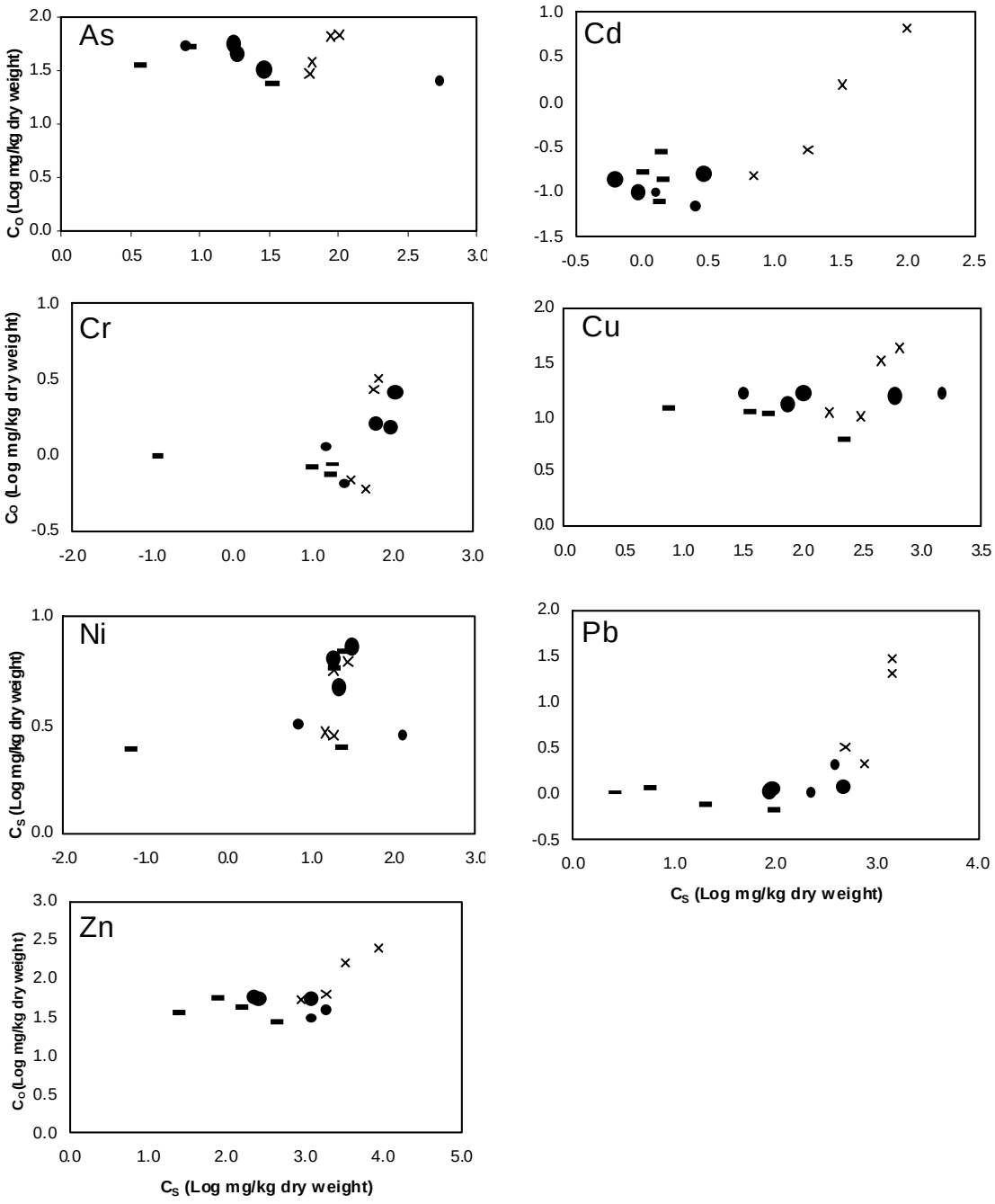

Fig. 1. Concentrations of metals in sediments (C_S) and in lugworms (C_O) for all the sediments studied. (-) sediments from Cádiz; (×) sediments from Cartagena; (•) sediments from Huelva; (●) sediments from Barcelona. All concentrations in mg/kg dry weight.

3.6 BioAccumulation Factors (BAFs) and Biota-Sediment Accumulation Factors (BSAFs)

The calculated BAFs and BSAFs were very variable among and between samples and elements (Table 6). The uncontaminated sand presented the highest BAFs due to the low chemical concentrations in sediments. Furthermore, the highest BAFs after exposure to the control sediment were calculated for the metals Cr, Cu and Pb, that reported higher concentrations in lugworms exposed to their home sediment than individuals exposed to the control sediment. This results may indicate the unsuitability of these control sediment as reference sediment for the evaluation of results from the dredged materials being assessed, although in this study it was still used for comparison purposes.

The mean BAF values were higher for As>Ni>Zn>Cu>Cd>Cr>Pb, which evidence the tendency of essential metals to present higher BAFs values due to the higher bioconcentration of these metals for essential functions. BAFs values higher than 1 were only found for As as the concentrations in lugworm tissues are higher than those in sediments. The highest sediment concentrations of this element, reported in Huelva, corresponded to the lowest BAF values while the highest values corresponded to the lower concentrations in sediments (CA1, CA3, CA4, B1 and B3). Similar considerations apply for the rest of metals and a general inverse relationship between concentrations in sediments and calculated BAFs was identified, with the lowest values for the sediments from Huelva H2 and H3, C2 and C4 in Cartagena, B4 in Barcelona and CA2 in Cádiz.

The correction for the organic matter content in sediments through the calculation of the BSAFs presented the highest differences with the BAFs values for the sediments with extreme contamination values (Table 6). Thus the BSAFs for the clean sandy sediment, which presented the lowest TOM content, were most often in the lowest range for all sediments tested, except for Cr, Cu, Ni and Pb that were still high. This correction still identified high values for the sediments CA3 and CA4 when compared to those calculated for other sediments. Although the mean BSAFs showed a similar trend among the metals studied to that identified through the BAFs, with the highest mean values for As>Ni>Zn>Cu>Cr>Cd>Pb, the BSAFs seem to be a better measure of the bioaccumulation potential from sediments than the BAFs as they are consistent with the level of metal bioaccumulation in lugworms within ports.

Nonetheless, if bioaccumulation potential is assessed as part of the characterisation process for dredged materials thus the results are processed to decide whether or not open water disposal is a suitable management option, a control sediment is recommended as a reference condition to evaluate the nature and extent of the bioaccumulation processes in the test organisms (Ruus et al., 2005).

Table 6

Bioaccumulation ratios for each sediment and for each of the metals studied (see text for explanaition).

Sediment		As	Cd	Cr	Cu	Ni	Pb	Zn
CA1	BAF	10.7	0.18	10.0	1.76	40.8	0.45	1.79
	BSAF	0.11	0.01	0.11	0.02	0.44	0.01	0.02
CA2	BAF	0.79	0.11	0.06	0.03	0.13	0.01	0.07
	BSAF	0.98	0.01	0.01	0.01	0.02	0.01	0.01
CA3	BAF	2.82	0.07	0.10	0.23	0.34	0.04	0.32
	BSAF	2.78	0.01	0.02	0.05	0.07	0.01	0.07
CA4	BAF	6.91	0.22	0.05	0.35	0.33	0.23	0.88
	BSAF	3.84	0.05	0.01	0.09	0.08	0.06	0.21
H2	BAF	0.10	0.04	0.05	0.01	0.45	0.01	0.02
	BSAF	1.71	0.01	0.01	0.01	0.05	0.01	0.01
H3	BAF	0.09	0.05	0.08	0.02	0.02	0.01	0.03
	BSAF	0.47	0.01	0.01	0.01	0.01	0.01	0.01
B1	BAF	3.19	0.11	0.02	0.18	0.34	0.01	0.22
	BSAF	0.50	0.01	0.01	0.01	0.01	0.01	0.01
B3	BAF	2.45	0.23	0.03	0.16	0.21	0.01	0.27
	BSAF	0.64	0.01	0.01	0.01	0.01	0.01	0.01
B4	BAF	1.11	0.06	0.02	0.03	0.23	0.01	0.05
	BSAF	1.66	0.01	0.01	0.01	0.04	0.01	0.01
C1	BAF	0.68	0.07	0.05	0.07	0.21	0.02	0.03
	BSAF	2.11	0.01	0.01	0.01	0.02	0.01	0.01
C2	BAF	0.59	0.02	0.01	0.03	0.19	0.01	0.03
	BSAF	1.02	0.01	0.01	0.01	0.02	0.01	0.01
C3	BAF	0.76	0.05	0.05	0.07	0.30	0.01	0.05
	BSAF	1.40	0.01	0.01	0.01	0.02	0.00	0.01
C4	BAF	0.47	0.02	0.02	0.07	0.15	0.01	0.06
	BSAF	0.85	0.01	0.01	0.01	0.01	0.01	0.01

We calculated the BAR values for each metal and for each sample considering the concentrations in individuals exposed to sediments CA1 (Fig. 2), which is considered a negative control of toxicity, although this reference condition should correspond to the proposed disposal site for the sediments being assessed. The highest BARs were calculated for Cd and Pb (BARs between 1 and 40 and 1 and 30, respectively) followed by

Zn>Cu>Cr≈Ni>As (BARs between 0.6 and 1.8). The values were most often higher than 1 as the concentrations were generally higher in lugworms exposed to the treatment sediments than in lugworms exposed to the control. This approach leads to a different prioritisation of contaminants and sediments from that obtained when using the BAFs and BSAFs, as the BAF and BSAF represent the proportion of metal that is bioavailable for the individuals of *A.marina* while the BAR evaluates the bioaccumulation in terms of final results without considering the contaminant concentrations in sediments.

3.7. Considerations on metal availability to lugworms

Bioaccumulation studies have been recommended to a limited extent in the context of navigational dredging throughout the countries applying an effect-based system, principally due to the difficulties on interpreting the results and the scarce information on the ecological relevance of the measured concentrations (PIANC, 2006). Nonetheless other countries such as the EEUU or Canada are applying bioaccumulation tests for regulatory purposes. The following five questions summarise substantially what needs to be address to make sound conclusions (Pedicord et al., 1997): 1)What is the toxicological importance of the contaminants whose bioaccumulation from the dredged material exceeds that from the reference material? 2) By what magnitude does the bioaccumulation from the dredged material exceed bioaccumulation from reference material? 3)What is the propensity for the contaminant to biomagnify within aquatic food web? 4) What is the magnitude by which contaminants whose bioaccumulation from the dredged material exceeds that from the reference material also exceeds the concentrations found in comparable species living in the vicinity of the proposed disposal site? And 5) For how many contaminants is bioaccumulation from the dredged material greater than bioaccumulation from the reference material? Thus suitable operational procedures would be those that give answer to these questions, although some knowledge on the fate and effects of the contaminants being assessed and case-specific criteria that reflect local conditions are needed. In particular it seems that the problems for data interpretation may be solved by using some reference conditions, which may change completely from one project to another.

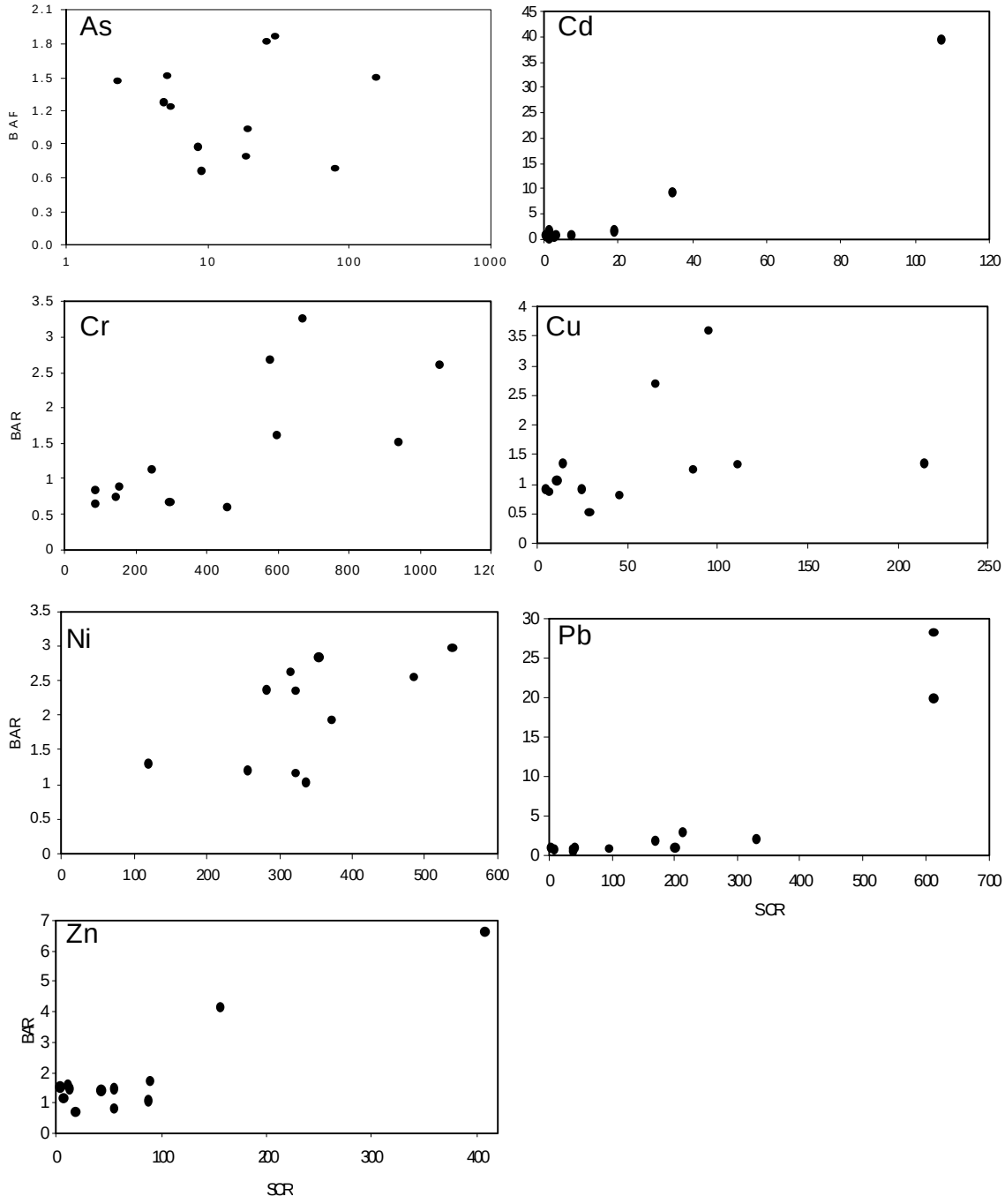

Fig. 2. Plot of Sediment Concentration Ratios (SCRs) and BioAccumulation Ratios (BARs) calculated for each treatment sediment for each compound (See text for calculation method).

In this study we used the control sediment CA1 as the hypothethic reference condition, thus the calculated BARs may be used as measurement endpoints to identify

enhanced metal accumulation in the individuals upon sediment exposure and the extent of the exceedance. This approach could be used to give answer to the questions mentioned above. Theoretical models have been also used to generate theoretical bioaccumulation potentials for organic micropollutants through the use of mean BAFs and/or BSAFs, although these models are not actually applied for inorganic contaminants. Concerning the use of BAFs and BSAFs, these ratios may be calculated from laboratory exposures and may be applied to assess the theoretical bioaccumulation potential later on. To address these compounds, bioaccumulation studies are recommended as they offer unique information on the actual bioaccumulation of especific compounds in especific benthic organisms (Ruus et al., 2005). Nonetheless they should not be used to assess contaminants that are metabolised or suffer some regulation or depuration process through sediment exposure as the bioaccumulation potential to other benthic organisms may be underestimated.

Different uptake studies have been performed to assess the bioaccumulation and depuration processes in lugworms although results are only reported for water-only exposures to different concentrations of single-spiked metals. These studies determined that *A.marina* accumulates the metalic contaminants studied in a dose dependent, non-linear manner when organisms are exposed to a range of concentrations (Geiszinger et al., 2002), but there are evidences on the little relevance of bioaccumulation from water phases in relation to sediment-bound contaminants, especially to polychaetes (Saiz-Salinas et al., 1997; Pesch et al., 1995; Ruus et al., 2005; Meador et al., 2005; Ugolini et al., 2004; Packer et al.; 1980). This is because the polychaete *Arenicola marina* is directly exposed to contaminants through gut desoption of sediment-bound compounds, as it feeds on the sediment where it burrows, and through exposure to the contaminants in solution in the water pumped through the funnel.

The importance of the sediment geochemical properties for predicting metal bioavailability and toxicity has been documented in the literature and it is because the proportion of fines, the TOM content of the sediments, the concentration of acid volatile sulphides and other factors interfere with available metals (i.e. Ruus et al., 2005). To further elaborate in the transfer of metals from sediments/pore waters to the tissues the results were investigated for some predictable interference of sediment properties and characteristics on the fate and effects of metals in the polychaete *Arenicola marina* exposed to several naturally contaminated sediments.

The correlation analysis did not indicate significant relationships ($p<0.05$) among the variables related to the sediment properties and the concentrations of metals in lugworms

(Table 7). The negative correlation coefficient points out some decreasing bioavailability of metals when increasing the proportion of fines and TOM and actually the correction for the organic content of the sediments (Fig. 3) evidenced interesting clusters of sediment samples by ports for some metals that did not appear when using the total sediment concentrations as exposure concentrations (Fig. 1). The sediments from Cartagena did not present major differences in the sediment properties but the sediments from Huelva and Barcelona formed one cluster per port when the Cd, Pb and Zn concentrations are corrected for TOM, which may evidence some contamination explained by the organic matter content that is not bioavailable to lugworms, thus not bioaccumulated. This would be in agreement with previous studies suggesting that organic matter may decrease the availability of metals such as Pb and Cu (Bernds et al., 1998; Bradham et al., 2006; Winner, 1984) and suggests some Cu and Pb co-disposed with a variety of anthopogenically-derived organic ligands, which reduce both its tendency to adsorb and the lability of these particulate forms (Turner, 2000). This hypothesis is supported by the lower bioavailability of Cu from the inner harbour sediments, that reported BAFs lower than those reported for intermediate sediment concentrations and a decrease in the proportion of bioavailable metal in relation to that desorbed in lugworm gut fluid in biomimetic experiments of 15% (Turner and Bishop, 2006; Chen and Mayer, 1999). While these results indicate that possibly gut desorption is the principal exposure route for Cu, it also indicates that TOM or other factor for which this variable accounts for determines the lower bioavailability of sediment-bound metals through this exposure route. Although this variable is a principal binding phase for some metals, the TOM content in the sediment also determines different amounts of sediment passing through the gut to meet nutritional requirements (Turner and Bishop, in press). The BAF values in our study were also in agreement with the proportion of BSA-available Cd and Zn relative to acid-extractable metal (around 2-26%), which indicates that gut desorption is also the principal route for the accumulation of these metals (Turner and Bishop, 2006), on the contrary the results of this mimetic approach for Pb indicate that other exposure route apart from gut fluids contribute in Pb accumulation in our sediments under laboratory simulation (Ruus et al., 2005).

Acid volatile sulfides (AVS) also control the accumulation of divalent metals in marine sediments (Ankley, 1996). The bioavailability of Cd and Zn is reduced for the polychaete *Neanthes arenaceodentata* by reducing the amount of dissolved metal on the water phase (Lee and Lee, 2005; Sundelin and Eriksson, 2001), but *Neanthes arenaceodentata* seems to accumulate Cd primary from sediment pore water. Other polychaete species are exposed

to sediment contamination through different exposure routes (Lee et al., 2000) depending on different biotic and abiotic factors (Selck and Forbes, 2004).

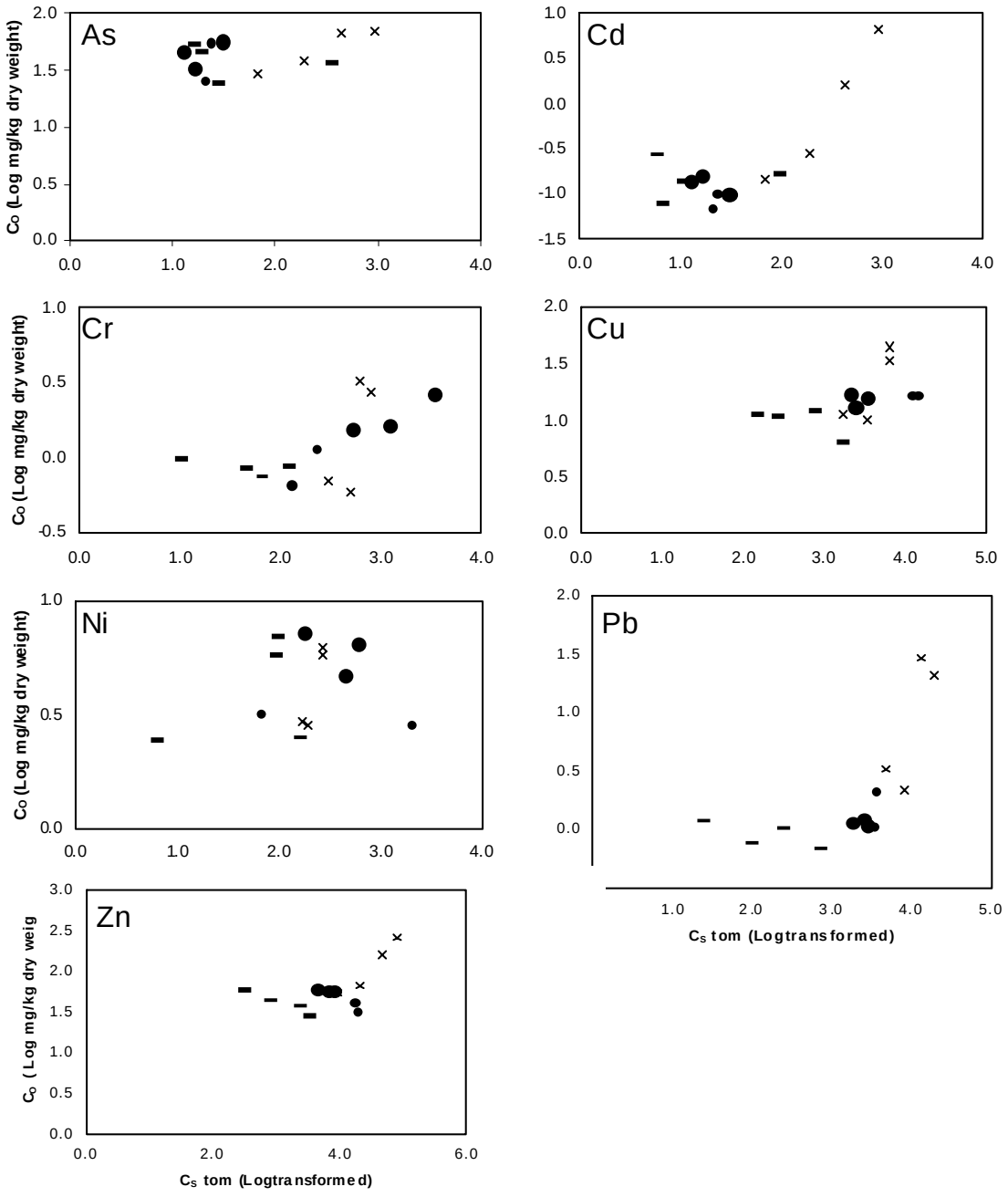

Fig. 3. Concentrations of metals in sediments normalised for the organic matter content ($C_S$tom) and concentrations in lugworms for all sites (C_O). (-) sediments from Cádiz; (×) sediments from Cartagena; (•) sediments from Huelva; (●) sediments from Barcelona. All concentrations in mg/kg dry weight.

The polychaete *Arenicola marina* feeds on large quantities of sediment in order to satisfy their nutritional requirements and simultaneously overlying water is pumped through the burrow in the opposite direction in order to oxygenate the gill filaments (Weston et al., 2000). Although AVS could determine the total amount of metals bioavailable to lugworms through water exposure, there are evidences on the digestion of metals bound to AVS by polychaete gut fluids (Turner and Bishop, 2006). Since fine grained anoxic sediments are supposed to be AVS-rich environments the slight negative correlation indicate the higher influence of TOM in the bioaccumulation of metals from sediments. Nonetheless the organic content and the proportion of fines are highly correlated due to similar patterns in sedimentary processes (Turner and Millward, 2002), which in turn could determine the results of the correlation analysis.

The only metals with positive coefficients were Ni and Cr. All our treatment sediments caused Ni bioaccumulation to concentrations corresponding to BAR values higher than 1, which is in agreement with results by Ruus et al. (2005) that identified some enhanced bioaccumulation in polychaetes *Nereis diversicolor* exposed to contaminated sediments in the laboratory. The metal Ni has not been studied to the same extent as some of the other essential metals though there seems to be an overall increase in body concentrations when increasing the exposure concentrations in water-only experiments (McGeer et al., 2003). Two possible reasons were argued by Ruus et al. (2005): 1) oxidation of metal sulphides, that would release Ni to interstitial water rendering it available for uptake either by absorption through the body surface or across the digestive tract, and 2) release from ingested sediment during the digestive process. Oxidation of metal sulphides can occur in the surrounding environment of burrowing polychaetes such as *Neanthes arenaceodentata* as argued by Pesch et al. (1995) but also in other burrowing polychaetes such as *Nereis diversicolor* (Otero et al., 2000) thus possibly for *A.marina* too. Nonetheless metal sulphides could be also oxidized when overlying water was changed. Most of the treatment sediments were fine-grained and sediment resuspension during water changes could not be avoided. This would also explain the lower bioaccumulation of Ni in lugworms exposed to sediment H3, with a higher proportion of sands thus resuspended to a lesser extent and subject to a lower oxidation of metal sulphides.

Table 7
Spearman rank correlation coefficients between sediment concentrations and concentrations of metals in lugworms (Co). Significant correlations at $p<0.05$ are marked with an asterisk.

	As	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**% fines**	**TOC**	**C_o As**	**C_o Cd**	**C_o Cr**	**C_o Cu**	**C_o Ni**	**C_o Pb**
C_o As	0.30	0.49	0.20	0.21	0.12	0.18	0.33	0.23	0.03	1.00					
C_o Cd	0.23	0.86*	0.34	0.30	0.23	0.47	0.58	-0.21	-0.24	0.63	1.00				
C_o Cr	0.31	0.62	0.30	0.43	0.15	0.46	0.56	-0.16	-0.22	0.61	0.78*	1.00			
C_o Cu	0.41	0.76*	0.34	0.43	0.14	0.52	0.62	-0.12	-0.26	0.79*	0.83*	0.90*	1.00		
C_o Ni	-0.04	0.23	0.47	0.17	0.52	0.11	0.20	0.44	0.62	0.56	0.40	0.51	0.52	1.00	
C_o Pb	0.51	0.92*	0.45	0.49	0.26	0.67*	0.74*	-0.21	-0.36	0.66*	0.90*	0.78*	0.90*	0.31	1.00
C_o Zn	0.32	0.90*	0.47	0.38	0.33	0.56	0.65	-0.13	-0.15	0.72*	0.95*	0.78*	0.90*	0.54	0.94*

A significant decrease in survival was caused by the sediments that actually caused an evident increase in the concentration of metals in lugworms thus lugworms seem to accumulate metals more efficiently under stressed conditions. This determines different bioaccumulation trends among metals. This is especially evident for the metals As and Cu, with the highest concentrations in the sediments from Huelva H2 but the highest concentrations in lugworms exposed to the sediments causing the highest toxic effects C1 and C3. The plot of concentrations in sediments-concentrations in lugworms (Fig. 2) showed an overall concentration-to-exposure relationship that evidences an slight accumulation over the range of sediment concentrations tested specially for As but also for Cu while there was not an straightforward relationship between concentrations in sediments and in lugworms. The highest BAFs and BSAFs were calculated for As as it is possibly an essential micronutrient for *A.marina* as for many animals and plants (Neff, 2002) and although its bioaccumulation through water-only exposure is further documented in the literature (Neff, 2002; Bernds et al., 1998; Everaarts and Saraladevi, 1996) and concentrations as high as 2000 mg/kg are reported in the literature for field collected organisms (Gibbs et al., 1983), some physiological regulation or detoxification of internal concentrations in benthic invertebrates can be achieved (McGeer et al., 2003; Neff, 2002; Rainbow and Dallinger, 1993).

Previous studies have also suggested the ability to regulate the essential metal Cu by the polychaete *Nereis diversicolor* at low, natural concentrations in sea water, with a breakdown point that represent a threshold for a clear bioaccumulation (Neff, 2002). The regulation of Cu have been also suggested from very high substratum concentrations (Bryan and Gibbs, 1987) although it is difficult in this study to elucidate if the lower concentrations in lugworms is due to the regulation or detoxification of metals by the lugworms or such variability is caused by the lower availability of sediment-bound metals. A breakwdown point that represent a clear bioaccumulation seems to appear, which is especially evident for Cd, Pb and Zn. For these metals "a visu" assessment allows to delineate some range of contamination at which a clear increased accumulation of metals in lugworms can be expected. For Cd this limit concentration is established around 30 mg/kg dry weight with a large variability in metal accumulation between 1.25 and 17 mg/kg which corresponds to the uncertainty area between the corresponding ERL/ERM and AL1/AL2 values (1.2/9.6 and 1.0/5.0 respectively). For Zn and Pb there is a clear break point at 1885 mg/kg and 90 mg/kg for Zn and Pb respectively, which is 0.5× the higher limit values for dredged material management (AL2s; Table 1). Although these results suggest the

possibility of developing SQGs to predict when bioaccumulation and toxicity are probable in lugworms, this approach seems not useful at lower sediment concentrations possibly due to the large variability on the availability of sediment-bound contaminants, which is determined by the high differences in the sediment properties and characteristics whithin and between ports.

This study has presented the results of laboratory simulation to assess the bioaccumulation potential of sediment-bound contaminants. The sediments represented a wide variety of sediment properties and characteristics affected by a wide variety of contamination sources. Because bioaccumulation is not an straightforward process, this study and other studies stress the importance of addressing especific contaminants in especific organisms such as the lugworm *Arenicola marina*. This is of special concern in the context of navigational dredging, where this information is used for to make a decision. If this type of studies are used for decision-making, worst case scenarios should be considered. Thus, the use of this species should be further studied concerning its relative sensitivity in this type of studies. Nonetheless, and considering that there are few or no evidences of biomagnification of these metals through food-chain (Neff, 2002; Bernds et al., 2003), the ecological relevance of the concentrations of contaminants in the tissues of benthic invertebrates should be investigated through validating studies with field collected organisms.

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Bioaccumulation of Hg, PAHs and PCBs from dredged sediments to the Lugworm (*Arenicola marina,* L.)[1]

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Abstract

Lugworms (*Arenicola marina*) were used to evaluate the bioaccumulation of contaminants in dredged sediments. Different sediments from Spanish ports were tested for bioaccumulation following the standard 10 day test and the organisms were analysed for mercury (Hg), organic mercury, 7 congeners of polychlorinated byphenyls (PCBs) and some polycyclic aromatic hydrocarbons (PAHs). The results showed that lugworms clearly accumulate the contaminants from sediments but with different bioaccumulation trends for each group and for some specific compounds. Lugworms bioaccumulated Hg when toxic effects were significant, although a relatively uniform Hg content was reported for a wide range of sediment concentrations, for which lethal effects were not observed. Bioaccumulation of PCBs was higher at the highest sediment concentrations, although both Hg and PCBs accumulation may be critically determined by the organic matter content or other factor for which it accounts for. In the case of the PAHs, no relationship was observed between concentrations in sediments and concentrations in lugworms, suggesting a more complex process of bioaccumulation. The results of this and other studies indicate the need for direct measures of concentrations in organisms directly exposed to the sediments being assessed, such as the polichaete *Arenicola marina,* to identify early increased bioaccumulation of these contaminants.

Keywords: dredged material characterisation, polychaetes, sediment quality, bioaccumulation

INTRODUCTION

Mercury, organic mercury, PCBs and PAHs have been further studied since they are liable to bioaccumulate and biomagnify and thus pose a risk to marine top predators (Stronkhorst, 2003). This should be taken into consideration in contaminated sediment and dredged material assessment frameworks, especially if the information retrieved is used for

[1] Chemosphere (Enviado)

decision-making and management purposes (PIANC, 2006; US EPA, 1991). Current routine practices for investigating the nature and extent of dredged material contamination involve determining the total content in sediments as an estimate of exposure, which assumes 100% of the total concentration is bioavailable, despite the actual bioavailability may be much lower. As for acute toxicity, specific issues may be encountered locally and regionally which determine that chemical analyses alone are not good predictors of bioaccumulation in a biological system, since the different physico-chemical properties of contaminants and sediments can affect bioavailability. Whereas this assumption is conservative in terms of being overprotective of human health and the environment, the true risk of these compounds may be overestimated and may result in lengthy and costly investigations (Bradham et al., 2006).

For a chemical to bioaccumulate it must be available for accumulation and, once it has entered the food chain, may be subject to biomagnification in top predators. Thus addressing questions concerning the potential of contaminants in dredged materials to move into the food chain and produce effects in organisms, above and beyond the borders of a disposal site, begins with assessing bioaccumulation potential. Various models have been described to predict the bioaccumulation potential, based on sediment chemistry and physico-chemical characteristics (e.g. organic carbon content). Although these models may be used for screening purposes they should be supported by following secondary biological tests in the laboratory (PIANC, 2006). Nonetheless there are still some difficulties to identify laboratory bioassays to address bioavailability by direct measures of bioaccumulation of specific compounds in specific organisms representative of the biota under risk.

This paper presents the results of laboratory tests with the marine benthic organism *Arenicola marina* (Polychaeta) to assess the bioaccumulation potential and bioavailability of Hg, PAHs and PCBs bound to dredged materials. This species offers important advantages for sediment toxicity assessment (Casado-Martínez et al., accepted) and has been widely used in different bioaccumulation studies (i.e. Kaag et al., 1998; Bernds et al., 1998). In addition it is widely distributed along the Spanish coast and is an important prey item that may contribute to the transport of contaminants to higher levels in the food chain. Previous results on the assessment of acute toxic effects in this species upon exposure to dredged sediments from Spanish ports showed the absence of mortality despite the high contamination of the sediments tested (Casado-Martínez et al., accepted), which indicates that this species may be a suitable test species to assess bioaccumulation. In this context the

lugworms were measured for total tissue concentrations with the main objective of investigating the suitability of this species for assessing bioaccumulation potential of sediment-bound Hg, PAHs and PCBs under laboratory conditions, especially in the context of navigational dredging. An exposure period of 10 days, shorter than the current 28 days used for bioaccumulation studies, was considered as a more cost-effective methodology according to previous studies performed to assess the bioaccumulation of different organic compounds in this polychate species (Bernds et al., 1998; Christensen et al., 2002; Geiszinger et al., 2002).

MATERIAL AND METHODS

Test sediments and chemical characterisation

Sediments from four ports were included in this study: Barcelona and Cádiz (named B# and CA# respectively), wich are important fishing and shipping ports near major centres of population; Cartagena and Huelva (named C# and H# respectively) are in a region hosting important sources of metals, and chemical and metallurgical factories, power plants, oil refineries, and a celulose factory (Huelva). In each port three to four sampling stations were selected to a total of thirteen stations representative of dredging operations and one sampling station (namely CA1) was selected as control sediment based on historical data (Riba et al., 2003). Sediments were collected with a 0.025 m^2 Van Veen grab from approximately the top 20 cm. After collection, the wet sediment was stored in clean polyethylene bottles at 4ºC and darkness.

Analytical methods were described in detail elsewhere (Casado-Martínez et al., 2006). With the exception of grain size, physico-chemical characterisation of sediments was done after drying at 40ºC for 24 hours. Grain size distribution followed UNE 103 101 and total organic matter content (TOM) was measured by loss-on-ignition at 550ºC and gravimetric determination as recommended for small dredged volumes. For Hg the cold vapour technique was used and was quantified using atomic absorption spectrometry. Polychlorinated biphenyl congeners (PCBs) and polycyclic aromatic hydrocarbons (PAHs) were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment and concentration and clean up with column chromatography. Determination of PCBs was made with gas chromatography with electron capture detection (GC-ECD) (EPA 8080) and PAHs were determined with HPLC with fluorescence detection (EPA 8310). All the analytical procedures were checked with

reference materials (MESS-1 for trace metals and NRC-CNRC HS –1 for organic compounds) and obtained concentrations that did not differ significantly ($p<0.05$) from certified values.

Test organisms, experimental set-up and tissue measurements

Individuals of *Arenicola marina* (Linnaeus, 1758) (lugworms) were collected in the Cantabric coast (north of Spain) by hand digging and were shipped refrigerated to the laboratory in the University of Cádiz in clean seawater. Once in the laboratory the organisms were immediately transferred into 20 L holding tanks with a 5 cm clean sediment layer and clean seawater for acclimatization to test conditions (temperature and salinity) for at least 1 week prior to tests. Before being used, organisms were transferred to 20 L holding tanks with clean seawater for at least 24 hours for depuration. The bioassay followed protocol by Thain and Bifield (2001). Sediments (2 L) were loaded into 12 L aquaria providing a layer of sediment 5 cm deep. After 12 hours approximately clean sea water (6 L) was added to each container and then exposure chambers were left to settle for 12 hours before aeration was started. After 12 hours of aeration five lugworms were placed in three replicate containers. No extra food was added to the test organisms and overlying water was renewed every three days or if any mortality was registered. After 10 d, surviving worms were placed in clean seawater to depurate for 24 h, then blotted dry and frozen at −80°C for later tissue analysis.

Bioaccumulation measurements

Hg

Total mercury (Hg_T) was measured from the liofilized organisms using an Automated Mercury Analyzer AMA-254 (Altec Ltd, Prague, Czech Republic) after drying (200ºC) and burning (700ºC) under constant oxygen flow. The analytical results were quality checked by analysis reference materials (DORM-2 and TORT-2) before and after each batch of samples. Obtained concentrations did not differ significantly ($p<0.05$) from certified ones.

Organic Hg

Organic mercury (Hg_{ORG}) was extracted from samples with concentrate HBr followed by extraction of the organic phase into toluene and back-extraction with an aqueous cysteine solution, according to the method described by Scerbo and Barghigiani (1998). Mercury in cysteine solutions was measured as described for Hg_T.

PAHs

Five sediments were considered to study the accumulation of PAHs in lugworms. Three sampling sites were located in the port of Barcelona: B4 in the inner harbour, B1 at the external part near open sea and B2 located along the harbour. In addition two sediments, C1 and C4 from Cartagena, were also studied. Tissues with a surrogate standard (Supelco solution: Acenaphthene-d10, Phenanthrene-d10, Chrysene-d12, Perylene-d12) were Soxhlet extracted with hexane/acetone (1:1) during 24h. Extracts were concentrated under a rotary evaporator, and fractionated with a silica:alumina (1:1) column. The second fraction eluted by 30 mL dichloromethane:hexane (1:9) and 40 mL dichloromethane:hexane (1:4), containing PAH compounds, was concentrated to 0.5 mL under a gentle stream of nitrogen before analysis. A blank was run up every 6 samples. For identification and quantification of PAHs the instrumental analysis was performed using Gas Chromatograph / Mass Spectrometry (GC/MS, Finnigan Mat, GCQ tm) operated in a Selective Ion Monitoring mode (SIM). A DB-5 (Argilent) silica fused capillary column (30 m x 0.25 mm inner diameter x 0.25 μm film thickness) was used with helium as the carrier gas at a constant flow rate of 1.5 mL/min. Splitless injection of 1 μL of the sample was conducted with an autosampler. The GC oven temperature was programmed from 70 ºC (0.30 min) then to 140 ºC (0.00 min) at 30 ºC/min before reaching 270 ºC at 3 ºC/min and held for 15 min. The injector and ion source temperatures were 270 ºC and 220 ºC, respectively. PAHs in the samples were identified by the retention time and the abundance of quantification ions/confirmation ions with respect to authentic PAH standards. Detection limits were 0.2 – 4.5 ng g^{-1} dry weight for PAHs. The average recoveries (n=7) of the analytical procedure were performed with certified reference material CRM 126-100 (RTC) and the recoveries data ranged between 75 and 120 %. All the results were expressed on a dry weight basis.

PCBs

Three sediments were studied for PCB accumulation in lugworms. The sediments corresponded to samples CA2 in the inner fishing harbour in Cádiz, B2 in the port of Barcelona and C2 in the port of Cartagena. The liofilized organisms were soxhlet extracted in n-hexane for 6 hours. The extracts were purified in Fluorisil column (1% H_2O) and elutes with hexane and dichlormethane/hexane. PCB congeners were determined using a Hewlett-Packard 5880A gas chromatograph with an electron capture detector and a capillary column (DB5, JW, 60 m). To identify and quantify each compound a mixture of PCB congeners (PCB-1, IOC-Kiel) was used. Lipid content was determined by the weight

of hexane extracted components. The analytical detection limit was 0.1 ng g^{-1} and the mean recovery was 93±16.2%.

Data treatment and analysis

For Hg, bioaccumulation was evaluated through the development of BioAccumulation Ratios (BARs) and Sediment Concentration Ratio (SCRs) using the results from a reference sediment (namely CA1):

$$BAR = C_{OS} / C_{OC}$$

where [C_{OS}] is the corresponding mean concentration of Hg in lugworms exposed to the sediment (S) and [C_{OC}] is the concentration of Hg in lugworms exposed to the control sediment. For comparative purposes sediment concentration ratios (SCRs) were calculated as follows:

$$SCR = C_S / C_C$$

where [C_S] is the concentration of Hg in the sediment (S) and [C_C] is the concentration of Hg in the control-sediment (C).

To evaluate the magnitude of bioaccumulation of organic compounds no comparisons between BARs and SCRs could be performed because none of the compounds were detected in the control-sediment. To have a measure of the bioaccumulation, BioAccumulation Factors (BAF) were developed individually or for the sum of compounds as follows:

$$BAF = C_{OS} / C_S$$

where [C_{OS}] is the mean concentration of the compound (C) in lugworms exposed to the dredged material (S) and [C_S] is the concentration of the compound C in the dredged material (S). In addition the Biota-Sediment Accumulation Factor (BSAF) was also calculated by normalizing the value for the organic matter content of the sediment as follows:

$$BSAF = C_{OS} / (C_S / f_{oc})$$

where [f_{oc}] is the sediment organic content.

RESULTS

Grain size distribution and organic matter content

The control sandy sediment presented the lowest TOM content. Sediment properties differed within and between ports, with TOM ranging from 7.19 to 24.33% (corresponding to C3 and CA4, respectively) and the proportion of fine sediments between 31.87 and 99.59 % corresponding to these same two sediments. Grain size and TOM varied a great deal inside ports, being organic matter preferentially accumulated in sediments deposited in inner parts (stations CA4, B4, C1 and H2). TOM content resulted significantly correlated with the % of fines ($r=0.84$, $p<0.05$; Fig. 1) although the inner sediments from Barcelona (B4) and Huelva (H2) presented a higher and lower TOM content than that expected from the fitting line.

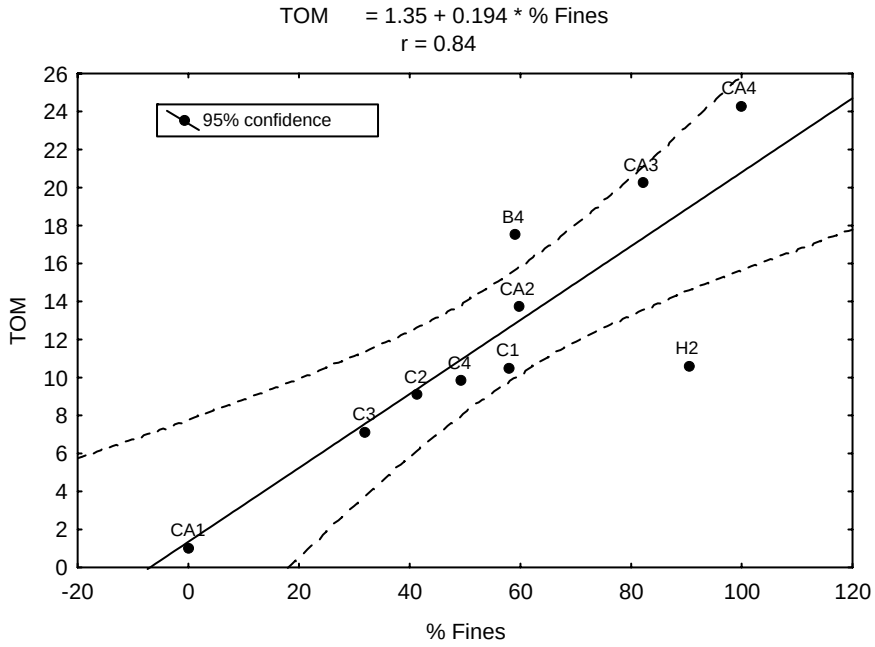


Fig. 1. Correlation between the proportion of fines and TOM content in the sediments assessed.

Hg

Concentrations in sediments

The concentrations of Hg in sediments ranged between 0.05 and more than 136 mg kg^{-1} dry weight (CA1 and C1, respectively; Table 1). The lowest Hg concentrations were reported in sediments from the port of Cádiz although the sediments from the inner

fishing harbor presented a higher enrichment, with intermediate Hg concentrations according to the limit values used in Spain for dredged material characterisation and management (1.98 mg kg^{-1} while limit values are 0.6-3.0 mg kg^{-1}). Similarly, the sediments from Huelva (H2) and Barcelona (B4), affected by different anthropogenic sources of Hg, also reported concentrations exceeding the lowest and highest limit concentrations of Hg for dredged material management (1.99 and 4.12 mg kg^{-1}, respectively). The sediments from Cartagena presented Hg concentrations one order of magnitude higher than the highest national limit value for sediments C2 and C4 (32.71 and 21.59 mg kg^{-1}, respectively), in the western part of the bay, and two orders of magnitude for sediments C1 and C3 (136.4 and 115.2 mg kg^{-1}, respectively). Even if it seems that the sediments from the inner harbours are enriched in fine particle sizes, TOM and Hg, a clear correlation could not be established between these three variables as the sediments from Cartagena presented the most conspicuous concentrations of Hg, not related to significant proportion of fines or TOM.

Concentrations in lugworms

Lugworms exposed to the control sediment presented concentrations of Hg in the lowest range for all sediments tested (0.05 mg kg^{-1} dry wt.). The exceptions were lugworms in CA3 and CA4 with 0.02 and 0.01 mg kg^{-1} dry wt., respectively. When exposed to the sediments from the inner part of this port (CA2), that presented a concentration in sediment of 1.98 mg kg^{-1} dry wt., lugworms accumulated higher concentrations of Hg (0.08 mg kg^{-1} dry wt.). In other ports the inner sediments also caused some increase in the concentrations of Hg in lugworms (0.11 mg kg^{-1} dry wt. for H2, 0.08 mg kg^{-1} dry wt. for B4). In sediments from Cartagena, accumulation of Hg in lugworms exposed to the lowest sediment concentrations was similar to low and intermediate sediment concentrations (0.12 and 0.14 mg kg^{-1} dry wt. for C2 and C4 respectively), while lugworms in sediments C3 and C1, with concentrations of Hg above 100 mg kg^{-1} dry wt., reported 1.98 and 10.52 mg kg^{-1} dry wt. respectively. The concentrations in lugworms after 10 days of exposure were less variable than concentrations in sediments (Fig. 3), although the rank of samples according to sediment concentrations (C1>C3>C2>C4>B4>H2>CA2>CA3>CA4>CA1) is in agreement with the rank of samples according to concentrations in lugworms (C1>C3>C4>C2>H2>B4=CA2>CA1>CA3>CA4).

Although organic Hg compounds were not measured in sediments, the accumulation of these compounds in lugworms was assessed after the 10 days of exposure to some of the samples tested (Table 1). The results showed a clear increase in the accumulation of

organic Hg in lugworms as the total Hg concentration in lugworms increases, with the highest values after exposure to sediments C1 and C3 (0.4 and 0.081 mg kg^{-1} dry wt.).In addition, the rank of samples according to the content of organic Hg in lugworms was the same as the one generated according to the concentrations of total Hg in lugworms.

Table 1

Results of the concentrations of Hg in sediments (C_S) and in lugworms (C_O) expressed as mg kg^{-1} on dry weight basis and calculated ratios for each of the sediments assessed (BAFs, BARs and SCRs; see text for calculation expressions). Concentrations of organic mercury in lugworms (Hg_{ORG}) are expressed as percentage of the concentration of total Hg in organisms (C_O).

Sample	C_S	C_O	BAF	BAR	SCR	Hg_{ORG}
CA1	0.05	0.05	1	-	-	-
CA2	1.98	0.08	0.04	1.6	39.6	-
CA3	0.28	0.01	0.04	0.2	5.6	-
CA4	0.05	0.02	0.40	0.4	1	-
H2	1.99	0.11	0.06	2.2	39.8	39.1
B4	4.12	0.08	0.02	1.6	82.4	39.5
C1	136.4	10.52	0.08	210.4	2728	3.8
C2	32.71	0.12	<0.01	2.4	654.2	39.4
C3	115.2	1.98	0.02	39.6	2304	4.1
C4	21.59	0.14	0.01	2.8	431.8	39.4

PAHs

Concentrations in sediments

The total PAHs concentrations in sediments ranged between 280 and 1800 mg kg^{-1} for B1 and C4 respectively (Table 2). The concentrations were higher at B4>C4>C1>B3>B1. In Barcelona the concentrations increased in sediments from inner parts as the proportion of fines and TOM increased. On the other hand, the singificant increase in PAHs in the sediments at the entrance of the port of Cartagena was not related to an increase in the proportion of fines and TOM. Generally the highest concentrations were present for high molecular weight compounds, namely pyrene, indene, fluoranthene, benzo(g, h, i)perylene, benzo(b)fluoranthene and benzo(a)anthracene. Although it was not possible to rely on quotients to evaluate the contribution of PAH sources in each station, the fluoranthene/pyrene ratio indicated a higher contribution of petroleum hydrocarbons in the inner sediments from Barcelona and at the entrance of the port of Cartagena.

Concentrations in lugworms

PAHs tissue concentrations were only determined in lugworms exposed to sediments B1, B3, C1, C4 and B4, with sediment concentrations for the sum of compounds of 280, 610, 910, 1240 and 1800 mg kg^{-1} dry wt., respectively while in lugworms exposed to these sediments were 640, 453, 443, 389 and 477 mg kg^{-1} dry wt., respectively (Table 2). These results determine a very different rank of samples: B1<B3<C1<C4<B4 according to the concentrations in sediments and C4<C1<B3<B4<B1 when considering the concentrations in lugworms. These results also determined that the highest BAF for the sum of PAHs was calculated for B1, which presented the lowest concentration in sediment but the highest concentration in lugworms (BAF=2.67), while for the rest of sediments BAF values ranged between 0.26 and 0.74. When considering the individual PAHs compounds, the highest tissue concentrations were reported for the PAHs benzo(b)fluoranthene, phenanthrene, pyrene and fluoranthene between 33 and 74 mg/kg while the rest of compounds reported tissue concentrantions between 20 and 40 mg/kg, except anthracene that reported the lowest tissue concentrations between 10 and 20 mg/kg.

Table 2

Results of the concentrations of PAHs in organisms expressed as μg kg^{-1} dry wt. N.D. means not detected

PAH[a]		B1	BAF[b]	B3	BAF	C1	BAF	C4	BAF	B4	BAF
ANY		46.65	--	30.88	--	30.16	--	27.08	--	31.52	--
ANA		40.80	--	27.70	--	27.05	--	24.34	4.87	28.64	5.73
F		37.14	--	25.84	--	24.80	--	22.46	--	26.07	1.74
P		74.68	14.94	51.56	1.29	50.19	1.00	44.77	0.56	52.56	0.44
A		19.44	--	13.22	0.13	13.08	--	11.75	0.12	13.58	0.45
FL		54.51	1.82	40.03	0.57	38.73	0.43	33.41	0.20	41.77	0.23
PY		56.97	1.90	41.87	0.52	41.77	0.46	35.56	0.25	48.75	0.20
BA		34.08	1.70	23.27	0.58	21.63	0.36	18.92	0.21	22.14	0.74
C		34.35	1.72	25.04	0.50	24.39	0.35	20.27	0.20	25.66	0.18
BBF		67.75	3.39	50.56	1.26	50.42	0.42	42.42	0.33	55.10	0.26
BKF		27.59	1.38	19.57	0.49	18.56	0.37	16.39	0.23	20.52	0.21
BAP		34.34	1.14	23.14	0.33	22.89	0.25	20.69	0.16	24.21	0.13
IN		37.04	0.93	26.06	0.33	25.07	0.19	23.41	0.16	27.45	0.12
DBA		36.07	0.36	24.08	0.24	23.81	0.60	21.34	0.53	25.35	0.51
BPE		41.00	0.82	28.44	0.41	27.70	0.23	25.72	0.18	29.94	0.16
Total	C_S	240		610		910		1240		1800	
	C_O	640.5	2.67	453.0	0.74	443.7	0.49	389.8	0.31	476.7	0.26

[a]ANY: acenaphtylene; ANA: acenaphtene; F: fluorene; P: phenanthrene; A: anthracene; FL: fluoranthene; PY: pyrene; BA: benz(a)anthracene; C: chrysene; BBF: benzo(b)fluoranthene; BKF: benzo(k)fluoranthene; BAP: benzo(a)pyrene; IN: indene; DBA: dibenz(a)anthracene; BPE: benzo(g.h.i)perylene.

[b]BAFs not calculated if concentrations were similar to the detection limits.

PCBs

Concentrations in sediments

Only the total concentrations for the sum of PCBs congeners were available, varying from 140 μg/kg dry wt. for CA2 and B2 and 468 μg/kg dry wt. for C2 (Table 3). Among these sediments the TOM content was more variable than the proportion of fines, ranging between 4.55 for sample B2 and 13.75 for CA2, with intermediate values for sample C2.

Concentrations in lugworms

While the concentrations of PCBs in sediments were similar for CA2 and B2 the concentrations in lugworms were 19.0 and 26.6 μg kg^{-1} dry wt., respectively. The concentration presented by sediment C2 was considerably high and conversely the concentration in lugworms exposed to this sediment was also higher than that presented by lugworms exposed to the other sediments (28.6 μg kg^{-1} dry wt.). This determine that calculated BAFs were 0.17, 0.23 and 0.03 for CA2, B2 and C2, respectively.

Table 3

Concentrations of PCBs in sediments (C_S) and organisms (C_O) expressed as the sum of the congeners measured in μg kg^{-1} dry wt. N.D. means not detected

Sample	C_S	C_O	BAF
CA2	144.9	19.0	0.17
B2	138.3	26.6	0.23
C2	468.2	28.6	0.03

Mortality of lugworms exposed to sediments

Lugworms in the control sediment showed a mean mortality of 15%. Sediments from Cádiz and Barcelona did not cause a significant increase in the lugworm mortality. The exception was sediment CA4, that resulted in a mean mortality of aproximately 20%, and the sediments from Huelva, with 25% mortality. The sediments from Cartagena presented very variable mortality results. Sediments C3 and C1, from the eastern part of Cartagena, were highly toxic inducing a mortality rate of 67 and 80%, respectively Sediments from the western bay (C2 and C4) did not decrease survival in lugworms in spite of being highly contaminated. A significant correlation ($r=0.87$, $p<0.05$; Fig. 2) between lugworm mortality and Hg concentration in sediments was observed, nonetheless as the

results of the control sediment show, 15 to 20% of mortality is not related with the pollutants analysed in this study. When the mortality results are represented according to the concentrations of Hg in lugworms the plot of all data fitted a logarithmic curve (R^2=0.71; Fig. 2). Organic pollutants (PAHs and PCBs) concentrations were not correlated with the mortality of lugworms.

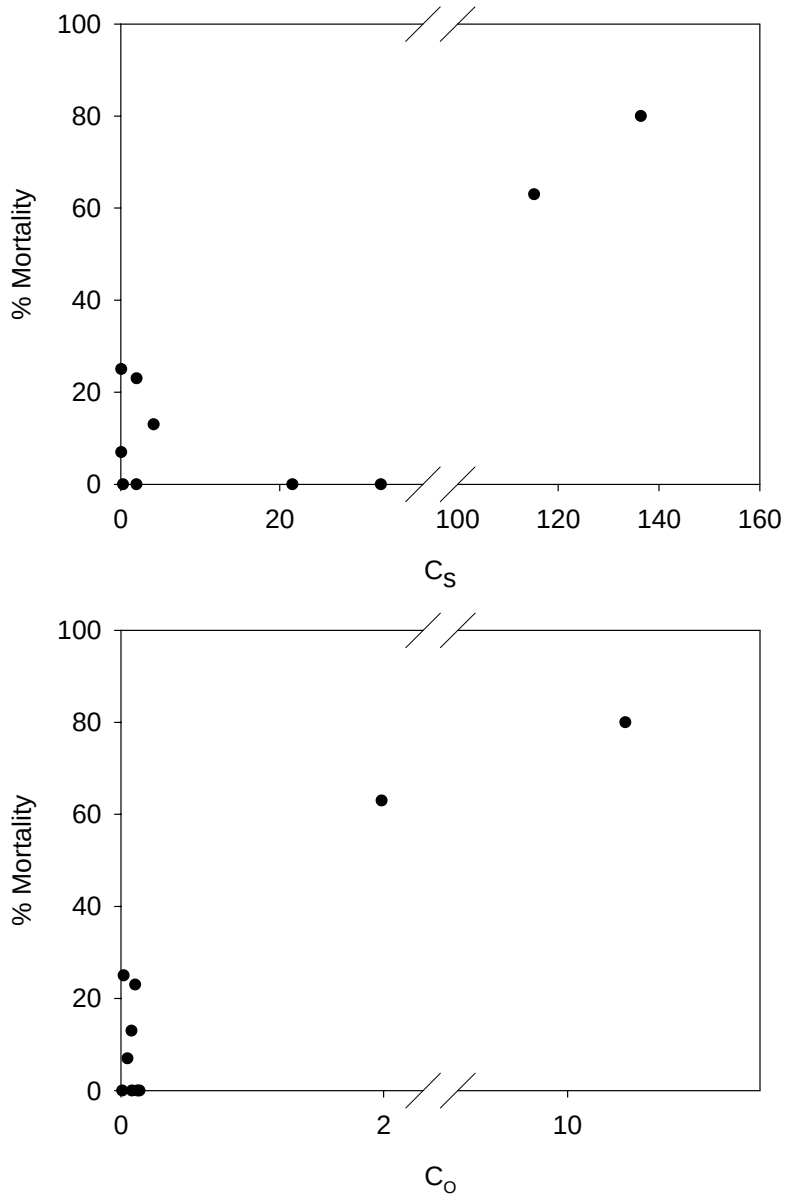


Fig. 2. Relationship between concentrations of Hg in sediments (C_S) and in lugworms (C_O) and mortality registered during the exposure to the sediments.

DISCUSSION

Hg

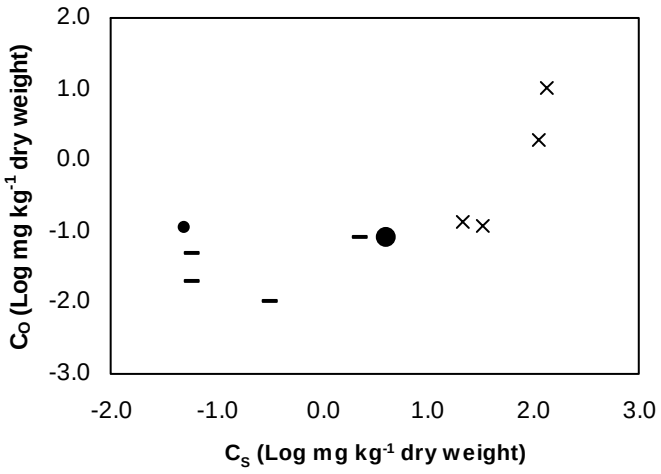
The bioaccumulation of total Hg in *Arenicola marina* depicted a clear non-linear trend, with slight increases in the concentrations in organisms over dramatic increases in sediment concentrations (Fig. 3). This same trend, also found for other metals, tend to substantiate the hypothesis of some physiological regulation of metals by *Arenicola marina* (Casado-Martínez et al., accepted), as previously reported for some essential metals such as Zn in different polychaetes (EEDP, 1986). Considering the mortality results as an indicator of the biological condition of the organisms being evaluated it seems that the threshold for significant toxic effects corresponds to a threshold for a dramatic increase in the accumulation of Hg by lugworms, which may be indicative of the disruption of this hypothetic biological system that regulates the internal concentrations of metals in the lugworms *A.marina*. At intermediate sediment concentrations the relationship between concentrations of Hg and mortality in lugworms was more variable than at extremely high sediment concentrations (Fig. 3), possibly due to the cocktail of contaminants in the sediments and the different sediment properties that determine the different bioavailability of sediment-bound contaminants.

The concentrations of Hg in lugworms exposed to low concentrations of Hg in sediments were in the lowest range for all samples, but the lowest concentration in lugworms did not correspond to the sandy sediment but to the uncontaminated fine grained sediments (CA3 and CA4). These results may be explained by previous results on differential mercury bioavailability and speciation in the various sediments types: water > sand > sand/mud > mud (Pesch, 1979), which are also related to different uptake rates on polychaetes (Jarvinen and Ankley, 1998). In the same line Muhaya et al. (1997) found that Hg concentrations in polychaete worms were not proportional to those in marine surface sediments due to the effect of sediment composition on the bioavailability of this metal, being the spatial distribution of Hg in sediments and in worms determined by the sediment composition and TOM content. In this study, spatial trends could be depicted in few ports due to the low number of samples considered in each area and according to a clear gradient in sediment concentrations. Previous studies on these sediments indicated that metals (Hg included) tend to complex with fine grained sediments, with organic matter as one of the key binding phases (Casado-Martínez et al., submitted). Considering that organic matter (e.g. humic/fulvic acids) present in sediments complexes Hg, and thus may result in less

bioavailability (Lu et al., 2005), the negative correlation between the proportion of fine sediments and TOM content, and the concentrations of Hg in lugworms is reasonable. This reduced bioavailability of Hg related to the sediment properties could not be observed on the calculated BAFs.

A new plot of Hg concentrations in lugworms versus concentrations of Hg in sediments corrected for the organic content explained some of the variability in the intermediate range of sediment concentrations (Fig. 3). The general trend in bioaccumulation is mantained according to the determining concentrations of Hg in Cartagena and not determining TOM content in these sediments. For the other sediments, the plot indicated that the similar tissue concentrations for some samples (B4, H2 and CA2) are explained by the TOM content or some other factor for which it accounts for. It is interesting to notice the CA2 and B4 are inner sediments while H2 reports also similar sediment physico-chemical properties. In the case of B4, this factor seems to be critical as the BAF value decreases to the BAF for C3, which is half of those calculated for CA2 and H2. The results from Cartagena formed a gradient in the range of high sediment concentrations. The sediments from the western part of Cartagena (C2 and C4) reported the lowest Hg concentrations in this port according to the lower influence of contaminant sources than in the eastern part (Casado-Martínez et al., 2006).

These two samples, that presented also a lower accumulation of Hg in lugworms reported the lowest BAFs values for all treatment sediments suggesting some decrease in the bioavailability of Hg. The BAF is one of the most simplified models for bioaccumulation, which considers a single-compartment model to predict partitioning between exposure medium (sediment) and biota. Empirical BAFs, generally calculated through laboratory experiments, are used to develop theoretical tissue concentrations as a method to screen for bioaccumulation. Our calculated BAFs varied among two orders of magnitude (three considering the uncontaminated sandy sediment) and evidenced some inverse relationship with the concentrations of Hg in sediments due to the determining results for low and high exposure concentrations. This is because the calculated BAFs are dependent on several biological and geochemical characteristics and, even more, dependent on the exposure concentrations considered for its development (McGeer et al., 2003). While these results agree with the unsuitability of the BAF approach as a stand-alone method for assessing the bioaccumulation potential from dredged sediments, it may still be useful as an screening method in terms of worst-case scenarios and serve for easy comparisons of metal bioavailability when testing in paralel.



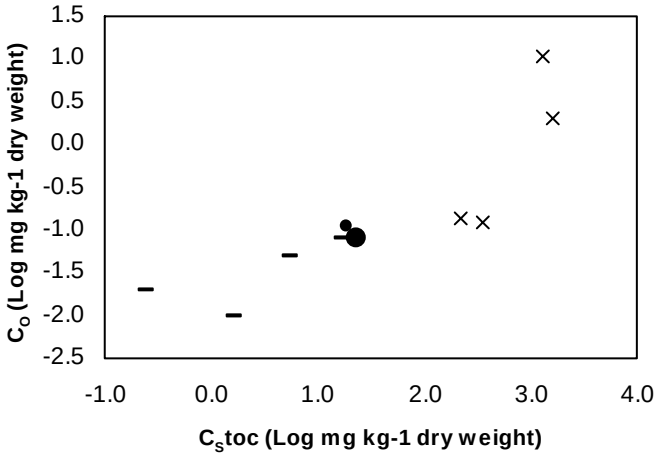


Fig. 3. Concentrations of Hg in lugworms and sediments for all sites: (-) sediments from Cádiz; (×) sediments from Cartagena; (•) sediments from Huelva; (●) sediments from Barcelona.

Although this study did not measure organic Hg concentrations in sediments, the concentrations of organic Hg compounds in lugworms showed a similar bioaccumulation trend to that obtained for total Hg. Muhaya et al. (1997) found simialr results for metilmercury, which is considered the form relevant for wildlife exposure and is much more toxic than inorganic Hg (Wolfe et al., 1998). The transformation of inorganic Hg by anaerobic sediment microorganisms produces MeHg, which is known to bioaccumulate at successive trophic levels in the food chain. If high trophic level feeders, such as birds and mammals, ingest sufficient MeHg in prey and drinking water, Hg toxicoses, including

damage to nervous, excretory and reproductive systems, result (Wolfe et al., 1998). Few studies on Hg speciation in relation to bioaccumulation in lugworms have been performed and, furthermore, it seems that there is a large variability in the proportion of Hg that is accumulated in its organic forms by different polychaete species: while in *Nereis diversicolor* MeHg accounts for 18% of the total Hg concentration (Muhaya et al., 1997), a 100% have been reported for the polychaete *Maldani sarsi* (Ali et al., 1997). No information is available for the lugworm *Arenicola marina,* but our results on whole organisms after 10 days of exposure ranged between 0.404 and 0.082 ppm registered upon exposure to sediments C1 and C3 respectively, 0.039 and 0.042 for H2 and CA2, and 0.049 and 0.055 ppm for C2 and C4. These concentrations represented a 3.8 and 4.1% of the total Hg in lugworms for C1 and C3, respectively while for the rest of sediments lugworms accumulated a 39.35±0.17% of total Hg in its organic forms. Although these results should be further studied, it seems worth mentioning that the plot of individual pairs of total Hg and organic Hg in lugworms fitted a line for the first group of sediments with a rate of transformation of 0.3877 with a R^2=0.99 (Fig. 4). Considering these results together with those obtained for the lethal effects, there is a breakpoint in the biological process that transforms inorganic Hg into organic Hg compounds in lugworms at the thershold for significant lethal effects. Before the rate of transformation/accumulation is 4% of the total Hg accumulated while after this thershold it turns into 40%, which agrees with the higher toxicity of organic Hg than inorganic Hg compounds.

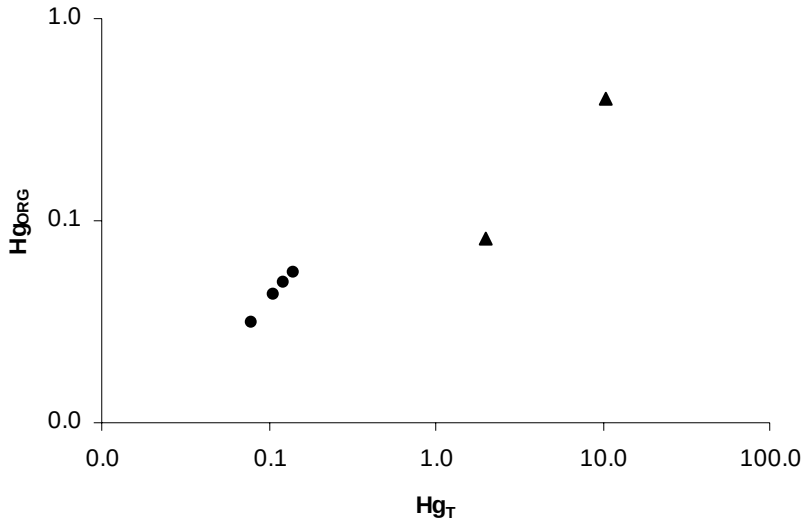


Fig. 4. Correlation between concentrations of total Hg and organic Hg compounds in *A.marina.*

PAHs

The results of bioaccumulation for the PAHs showed a slight negative relationship between the concentrations of PAHs in sediments and in lugworms. Moreover the comparison of sediments within each port did not show significant trends. Nonpolar organic chemicals such as PAHs have a low affinity for the water phase and high for poorly soluble organic phases such as tissues of aquatic organisms, organic particles or colloids. However marine sediments are an extreme heterogeneous mixture of different chemical composition and grain size of the sediment, which may result in a very different fate of sediment-bound chemicals (Neff, 2002). Among the different attempts to normalize the bioavailability of PAHs to cancel out the effect of some property of the sediment, the normalization to the concentration of total organic carbon in the sediment has been further discussed in the literature (Ruus et al., 2005; Timmermann and Andersen; 2003). However, the application of this correction to our results did not clarify the relationship between concentrations in sediments and in lugworms and the inverse correlation prevailed (Fig. 5).

In relation to the BSAFs a mean value of four is recommended for the calculation of the Theoretical Bioaccumulation Potential by the EPA (US EPA, 1991). This is about one order of magnitude higher than most of the calculated BSAF in this study (B1=0.81; C1=0.51; B4=0.46; B3=0.35; C4=0.22). Thus our results are more in agreement with those reported in MacFarland (1995) that stressed the overestimation of the bioaccumulation to benthic invertebrates when using the standard value of 4 compared to those found in laboratory experiments or field-collected organisms. This same report suggested that bioaccumulation may not be a linear function of organic carbon-normalized PAHs concentrations because a large fraction of these compounds may not be readily capable of desorption. The dominance of pyrogenic PAHs in the sediments may hamper the comparison between stations because a great proportion of the PAHs may be associated with the soot fraction in the sediments rendering them to be unavailable (Kaag et al., 1998a). Soot/water partitioning coefficients for phenanthrene and fluoranthene in sediments from Boston Harbor are about two orders of magnitude higher than the predicted sediment organic carbon/water partitioning coefficients (Koc), indicating very strong binding of the PAHs to the soot particles (Neff, 2002). A fluoranthene/pyrene ratio larger than 1 or a phenanthrene/anthracene ratio smaller than 10 indicate that the PAHs originated from pyrogenic processes (Benlahcen et al., 1997). Due to similar concentrations of anthracene in sediments to the detection limit for this compound the development of these quotients only considered a value for fluoranthene/pyrene >1 for C4 indicating a

high contribution of pyrogenic PAHs in this station, which is in agreement with the lowest bioavailability of sediment-bound PAHs according to the lowest BAFs. This approach is not useful in other cases possibly due to the high complexity of to different influence of point sources of petrogenic PAHs such as accidental oil spills or discharges from tankers and ships.

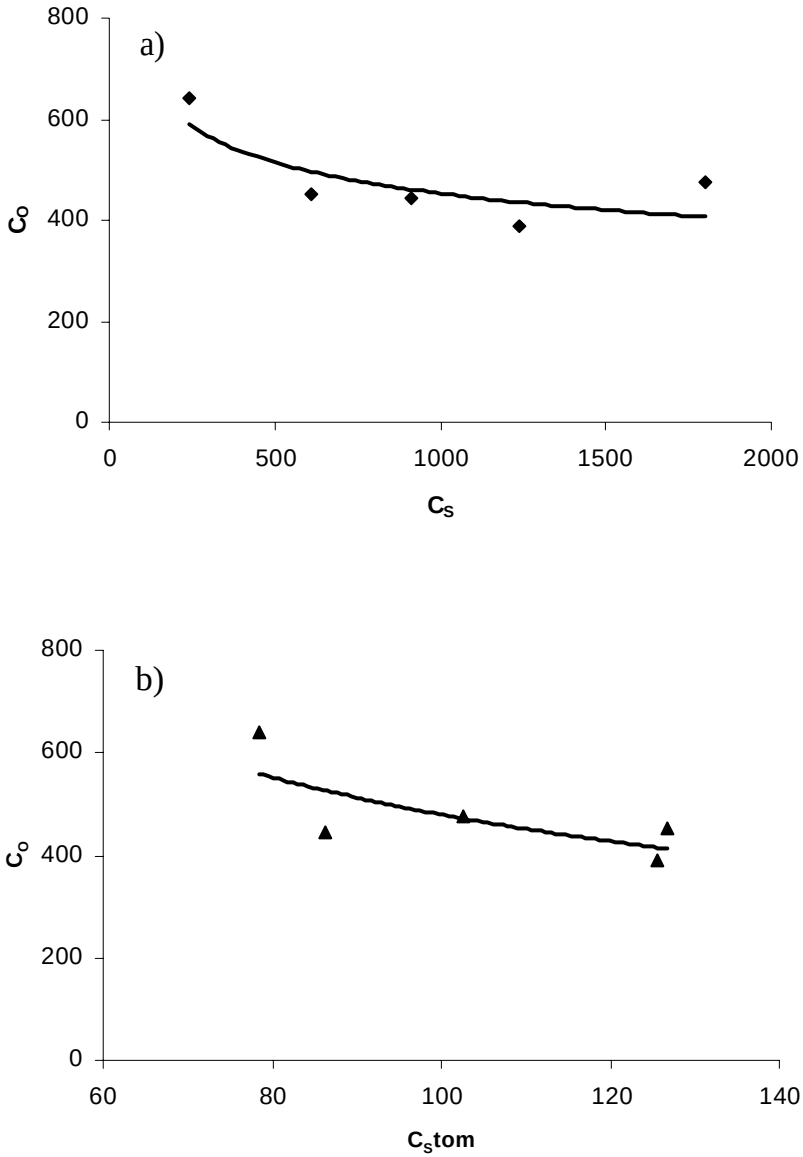


Fig. 5. Concentrations of PAHs in lugworms (C_O) and in sediments (C_S) before (a) and after the correction for the organic content of the sediments (b). All concentrations expressed as mg kg^{-1} d. w.

The results of indivual PAH compounds are in agreement with previous results on *A.marina* exposed to harbour and reference sediments that reported higher tissue concentrations after exposure to reference sediments for fluoranthene, pyrene, chrysene, benzo(e)pyrene and benzo(b)fluoranthene while exposure to harbour sediments enhanced the bioaccumulation of anthracene, benzo(e)pyrene, benzo(a)anthracene and chrysene. In our study, the sediments only accumulate anthracene and benzo(a)anthracene from sediment B4 that is actually in the inner harbour (notice the higher BAFs in Table 2), although the highest accumulation was registered for phenanthrene, fluoranthene, pyrene, benzo(b)fluoranthene and dibenzo(a)anthracene. These results are also in agreement with previous experimental results on *Nereis diversicolor* exposed to harbour sediments (Ruus et al., 2005). Furthermore this could be partly explained by previous results on enhanced solubilization for PAHs such as phenanthrene and benzo(a)pyrene in *A.marina* gut fluids in relation to predictions based on equilibrium partitioning theory and stressed the importance of the digestive pathway for hydrophobic organic contaminant exposure and bioaccumulation in deposit feeders, for which ingested sediment can be the primary source for the bioaccumulation of these contaminants (Voparil and Mayer, 2000; Weston et al., 2000). In addition, our calculated BAFs for individual compounds were in the range of other studies, for instante Meador et al. (1995) found the highest BAF for pyrene (BAF=0.4) while Ruus et al. (2005) reported a value of 0.20.

PCBs

The results for the three sediments studied showed a slight direct relationship between the concentrations of PCBs in sediments and in lugworms, as the highest concentrations of total PCBs in lugworms was associated to the highest sediment concentrations (Fig. 6). This accumulation trend was generally seen for all congeners, with the highest concentrations corresponding to the highest exposure concentrations (Fig. 7). The exceptions were the compounds CB-44, with a considerably higher concentration in lugworms exposed to sediments B2, although the absence of data of concentrations of individual congeners in sediments does not allow to evaluate this increased value. Congeners number 153, 187, 149 and 138 reported the highest concentrations in lugworms, which is in agreement with previous results reported for *N.diversicolor* exposed to harbour sediments (Ruus et al., 2005). Nonetheless our calculated BAFs were slightly lower than that found in the literature for different polychaetes (Ruus et al., 2005; Meador et al.,1997; Jonker et al., 2004). The possibility that steady-state concentrations were not

reached for these compounds may be considered due to the short exposure period compared to the traditional 28 days exposure, although previous studies have reported similar BAFs for other benthic organisms such as *Macoma nasuta* and *Mya arenaria* after exposure in standard sediment bioaccumulation studies (Boese et al., 1997; Lohmann et al., 2004). In this sense, the absence of data for reference conditions do not allow to make conclusions on the extent of the exceedance of bioaccumulation and the ecological significance of the measured concentrations.

The results allows to study the predictability of bioaccumulation of PCBs compounds in relation to sediment concentrations. The plot for the sum of congeners in lugworms versus the concentrations in sediments determined a clear lower bioaccumulation from sediment B2 than that expected from sediment concentrations, which was partially explained by the organic carbon content of the samples. The correction for the organic content improved the predictability of bioaccumulation thus the BSAF could be a better predictor of the actual bioavailability of PCBs. The Theoretical Bioaccumulation Potential calculated for screening purposes by the EPA establishes a mean BSAF value of 4, which is one-two orders of magnitude higher than the values found in this study. Nonetheless it is worth mentioning that many of the congeners that occur at higher concentrations in lugworms exposed to harbour sediments are in the group of congeners considered most environmentally threatening (Meador et al., 1997).

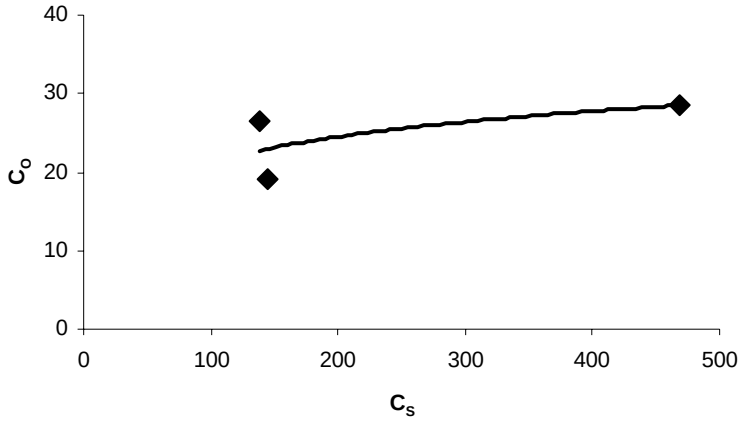


Fig. 6. Concentrations of PCBs in lugworms (C_O) and in sediments (C_S). All concentrations expressed as μg kg^{-1} d. w.

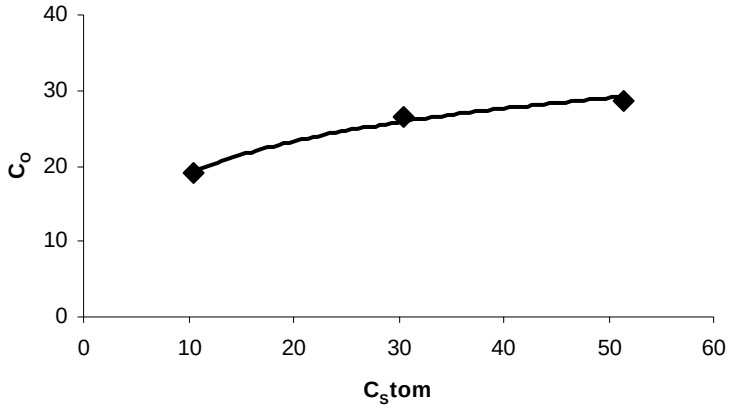


Fig. 6. Concentrations of PCBs in lugworms (C_O) and in sediments after the correction for the organic content of the sediments ($C_S$tom).

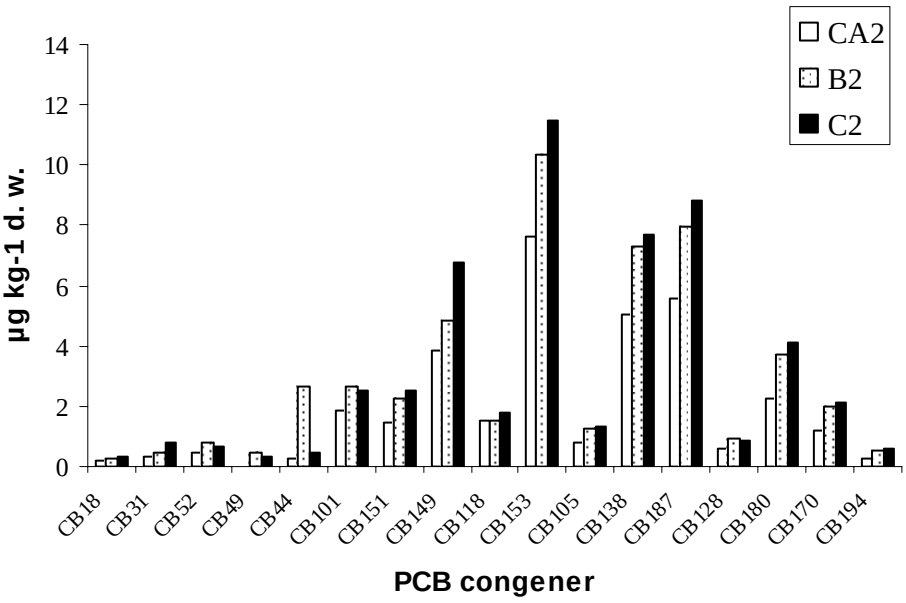


Fig. 7. Concentrations of PCBs congeners (#18, 31, 52, 49, 44, 101, 151, 149, 118, 153, 105, 138, 187, 128, 180, 170, y 194) in *A.marina* exposed to the sediments studied.

CONCLUSIONS

Different agencies such as the US EPA or Environment Canada recommend performing laboratory tests for the assessment of bioaccumulation potential in the context of navigational dredging. Five questions resume substantially what needs to be address for decision-making (Casado-Martínez et al., submitted), considering the number of compounds and the magnitude in which bioaccumulation from dredged material exceed

bioaccumulation from a reference material, and in relation to the toxicological importance and the propensity for the contaminant to biomagnify within aquatic food web. All these three types of compounds seem to experience a higher bioaccumulation in lugworms upon exposure to harbour sediments, although each group presented different bioaccumulation trends. The concentrations of Hg in lugworms do not increase over large increases in sediments, with a break point for a dramatic incorporation of total Hg and organic Hg compounds in the range between the SQGs in use for dredged material characterisation and management (Casado-Martínez et al., 2006). In relation to the organic compounds, the results of PCBs fitted a logarithmic curve while for PAHs, levels in lugworms were not correlated to sediment concentrations suggesting a more complex process of bioaccumulation.

The organic matter content seems to be a determining factor in the bioaccumulation of PCBs and Hg but this correcction did not improve on the predictability of the bioaccumulation from dredged sediments, which was one or two orders of magnitude lower than the standard theoretical bioaccumulation potential used in regulatory frameworks (McFarland, 1995). According to the high toxicological importance of these contaminants, which biomagnify and have deletereous effects at low concentrations, any increase in the concentrations in lugworms should be further considered in the regulatory process for dredged material management (PIANC, 2006).

Acknowledgements

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Bioaccumulation of metals in clams exposed to dredged sediments.

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ABSTRACT: Juvenile clams of the species *Ruditapes philippinarum* have been used to assess the bioaccumulation potential from dredged sediments upon 28 days of exposure in the laboratory. The contaminants assessed included As, Cd, Cu, Hg, Ni, Pb and Zn. The results indicate that clams ready accumulate higher concentrations of these compounds when exposed to higher sediment exposure, except for As. The correction of the sediment concentrations for the organic matter content reduced the variability of the results, especially in the case of Cu, but this factor seems not determining for other compounds such as Pb or Hg. The results for this species were less variable than previous results on *Arenicola marina* exposed to harbour sediments possibly due to the longer exposure period considered for the clams, or it may be also caused by the higher complexity of the bioaccumulation process of metals in this deposit-feeder than in clams.

Keywords: *Ruditapes philippinarum*, sediment quality assessment, dredged material, bioavailability

Assessing the bioaccumulation potencial of sediment-bound contaminants is a fundamental part of the characterisation needed to complete a proper environmental quality assessment framework for contaminated sediments and dredged material (PIANC, 2006; Chapman and Anderson, 2005). While biomagnification of metals is not still clear, standard 28-days bioaccumulation bioassays are recommended in the case there are evidences that such processes may occur. Among the recommended test organisms, different species of polychaetes and bivalves are preferred (PIANC, 2006). Bivalve mollusks have been identified as suitable indicators of sediment contaminants, especially metals, and it fulfils many of the criteria set down for species selection in toxicity testing: it is available all year round because it is a commercial species, it is easy to maintain in the laboratory, economically relevant and has a wide geographical distribution. Different species have been used, such as *Scrobicularia plana* (Byrne and O'Halloran, 2000; 1999; Riba et al., 2004a; 2003), *Macoma balthica* (Duquesne et al., 2004), *Tapes decussatus* (Mariño-Balsa et

al., 2003) or *Mya arenaria* (Phelps, 1990). This paper sumarises the results of bioaccumulation experiments performed on 19 sediments from Spanish ports on juvenile clams *Tapes semidecussatus* or *Ruditapes philippinarum* as this soft sediment dwelling mollusk is also known. This species is of commercial importance in Spain and has been used previously for sediment toxicity assessment (Casado-Martínez et al., 2006a; Riba et al., 2004b). The results are studied for general trends in the bioaccumulation between and within ports, and the possible use of this methodology for dredged material characterisation and management is evaluated in relation to previous results on the polychaete *Arenicola marina*.

Sediments were sampled at different Spanish ports: Pasajes, Bilbao, Cartagena, Barcelona, Huelva and Cádiz (PA#, BI#, C#, B#, H# and CA# respectively). At each port three to four sampling stations were selected in representative dredging sites. In each sampling site sediments were collected with a 0.025 m^2 Van Veen grab from approximately the top 20 cm of the bottom sediment and were brought to the laboratory and stored at 4ºC and darkness prior to analysis. Grain size distribution followed UNE 103 101 and total organic carbon (TOC) content was estimated by loss of ignition (LOI) at 550ºC and gravimetric determination as recommended for small dredged volumes. Metals were determined in microwave acid-digested samples (HNO_3 and aqua regia in a proportion 1:3) in Teflon vessels and adjusted to volume with boric acid 5.6%. For Hg the cold vapour technique was used and for As hydride generation, and both quantified using atomic absorption spectrometry. The concentrations of Cd, Pb, Cu, Zn and Cr were determined using flame or furnace atomic absorption spectrometry, depending on the metal content. The complete information on sample processing and analysis has been reported previously elsewhere (Casado-Martínez et al., 2006b).

For the bioassays, clams were obtained from a commercial hatchery and were acclimated to laboratory conditions in clean sea water for at least two weeks before the test was started. During this period animals were fed a mixture of different species of micro algae (*Tetraselmis chuii, Isochrysis galvana* and *Chaetoceros gracilis)* and were maintained in open water system. Test parameters and conditions have been reported in Casado-Martínez et al. (2006a). Biefly sediments were added to the test chambers to have approximately a 5 cm layer and clean sea water in a relationship 1:3. Test chambers were aerated at least 12 hours in advance to the addition of the organisms and then 40 organisms were randomly selected and added to each replicate. After the 28 days of exposure the organisms were sieved and placed in clean sea water for 24 hours to empty their guts. Organisms were pooled and

stored at -80ºC prior to liofilization and further tissue analyses. The analysis of metal content followed the methodology by Martín-Díaz et al. (2006). Briefly, the liophilized organisms were pooled and tissues were extracted with H_2O_2 and HNO_3 at 95 ºC for 45 minutes. After digestion, the solution was brought to 10 ml volume with deionised water and analytical determinations of metals were performed by inductively coupled plasma mass spectrometry. Each digestion included at least one blank treatment and one standard reference material (DORM-1 or TORT-2).

The sediments assessed presented very variable properties and characteristics within and between ports. The sediments were principally muds except sediment CA1, a typical sand with low TOM content. The rest of sediments reported a proportion of fines between 30 and 99% and TOM content between 4.55 and 24.33 g/kg (Table 1). Even if spatial variations could not be observed due to the little number of samples per port, these two variables were highly correlated (r=0.79). The sandy sediment, sampled in an intertidal area in the bay of Cádiz, was far from contamination sources and reported all concentrations below the lower ALs. The rest of sediments presented a very variable contamination in terms of chemical mixtures and concentrations, and comprised the three categories established for dredged material (Casado-Martínez et al., 2006a). Even if fine grained sediments, rich in organic matter content were generally associated to contamination, there was no significant relationship between the sediment properties and the contaminant concentrations due to the determining results in some ports under study. The sediments from Huelva presented the most conspicuous concentrations of As and Cu while Cartagena was affected by high concentrations of Hg, Cd, Pb and Zn.

Only the sediments from Huelva caused a significant decrease in survival and some delayed burrowing activity, while the rest of sediments did not cause neither lethal nor sublethal effects. It did not allow to collect enough organisms for tissue measurements, thus results for the sediments collected in Huelva and those for C1 were just included for comparison purposes but not to delineate the general trends in bioaccumulation. The correlation analysis evidenced that there was no relationship between the chemical load of the sediments and the toxic effects measured except for As and Cu (Casado-Martínez et al., submitted). As the As and Cu concentrations varied with the proportion of fines in the port of Huelva, the toxic responses were also correlated with this sediment property.

Table 1. Results of the physico-chemical properties of the sediments. All concentrations expressed as mg L^{-1} d.w, except TOM in g kg^{-1}.

	% sand	% fines	TOM	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
CA1	99.77	0.04	1.07	3.42	0.92	0.10	6.98	0.05	0.06	2.28	21.27
CA2	40.42	59.53	13.75	30.77	1.32	14.94	202.80	1.98	20.14	86.90	378.25
CA3	17.80	81.90	20.30	16.61	1.23	8.43	46.76	0.28	16.90	17.61	135.50
CA4	0.38	99.59	24.33	7.81	1.25	14.22	32.07	0.05	21.25	5.14	65.67
H1	9.71	90.22	20.27	840	4.35	32.89	1938	2.38	34.57	383.10	2458
H2	9.60	90.21	10.64	531	2.50	24.10	1497	1.99	7.10	384.7	1857
H3	56.02	43.95	6.30	273	1.32	8.13	772	1.20	129	217.6	1176
B2	57.92	36.58	4.55	21.19	1.52	103.70	159.7	1.12	29.12	103.5	424
B3	42.13	53.98	4.81	18.56	0.62	59.53	102.1	1.15	22.24	91.9	219.7
B4	39.89	58.70	17.56	28.99	2.88	93.86	601.1	4.12	32.30	455.3	1165
C1	38.24	57.81	10.54	101.5	98.49	66.64	665.9	136.40	29.04	1397	8661
C2	53.59	41.19	9.12	64.71	17.47	45.61	313.4	32.71	15.33	748.3	1885
C3	67.20	31.87	7.19	88.00	31.88	57.57	453.3	115.20	19.32	1397	3310
C4	50.01	49.10	9.87	62.55	6.79	29.48	171.1	21.59	19.32	486.7	900.8
BI1	20.28	77.33	14.81	67.26	2.00	18.27	102.6	0.74	26.39	147.5	476.1
BI2	14.48	47.40	15.07	104	2.00	23.11	204.10	1.43	32.00	285.9	777.5
BI3	6.22	93.59	16.73	21.71	0.04	3.48	23.03	0.18	15.72	40.7	122.35
PA1	28.87	70.29	14.43	39.13	0.68	26.73	158.10	1.07	33.49	293.7	1085
PA2	5.08	91.24	18.47	28.86	0.70	23.42	167.10	1.29	28.48	246	763
PA3	38.53	59.65	19.81	23.78	0.04	18.61	162.50	1.36	19.61	154.9	576

The results of the measured concentrations in clams is summarised in Table 2. The highest concentration in tissue were reported for the essential metals Zn, in the range of 50-70 mg/g and Cu and As at concentrations 9-11 and 7-9 mg/g respectively, and at lower concentrations, Ni. The lowest concentrations were reported for the non-essential Hg, Cd and Pb.

Table 2. Measured concentrations in clams after exposure to the sediments for 28 days. All concentrations expressed as mg L^{-1} d.w.

Sample	Ni	Cu	Zn	As	Cd	Hg	Pb
CA1	3.24	12.06	74.12	10.58	0.23	0.27	1.32
CA2	1.81	12.19	57.60	9.40	0.23	0.29	0.59
CA3	3.47	13.14	56.17	9.66	0.20	0.38	0.76
CA4	3.65	9.67	51.33	10.43	0.22	0.35	0.59
B2	1.42	24.48	188.51	8.63	2.08	0.65	6.61
B3	0.28	29.52	196.10	7.46	4.14	0.78	16.49
B4	1.52	15.06	101.63	9.99	0.87	0.66	12.89
C2	1.98	10.10	71.50	10.17	0.40	0.22	3.92
C3	3.00	6.31	47.92	9.07	0.21	0.30	1.28
C4	2.30	14.05	61.40	8.33	0.28	0.54	4.24
BI1	2.00	10.08	64.61	8.72	0.25	0.25	3.37
BI2	3.27	10.26	66.56	9.17	0.31	0.28	1.81
BI3	3.24	12.06	74.12	10.58	0.23	0.27	1.32
PA1	1.81	12.19	57.60	9.40	0.23	0.29	0.59
PA2	3.47	13.14	56.17	9.66	0.20	0.38	0.76
PA3	3.65	9.67	51.33	10.43	0.22	0.35	0.59
H1	2.34	12.22	67.58	10.45	0.60	0.34	2.71
H2	1.86	16.71	63.05	8.79	0.29	0.31	2.61
H3	2.29	13.60	68.71	8.73	0.31	0.39	3.05
C1	1.72	6.44	56.26	9.32	0.30	0.46	2.14

The plot of concentrations in sediments and in lugworms identified a clear correlation between the concentrations measured in clams and the concentrations in the sediments, except for As and Ni (Fig. 1). In the case of Ni, this variability is associated with a group of sediments that cause some enhanced bioaccumulation possibly related to some oxidation of metal sulphides during water exchange (Casado-Martínez et al., submitted). The rest of concentrations measured in clams appeared well correlated with the corresponding sediment concentrations and evidenced a clear increase in the bioaccumulation of this metal at higher exposure concentrations. For Pb and Zn, the plot of results showed a direct relationship between concentrations in sediments and in clams while for Cd, Cu and Hg the results at high exposure concentrations are lower than those resulted from a simple linear regression. These relationships identified the higher slopes for Cd, followed by Zn, Cu and Pb (0.0475, 0.0216 and 0.011; Table 3). The results of Hg were determined by the highest concentrations (0.0044; R^2=0.5832) as the plot of data may be also adjusted by a logarithmic line. These results are generally in agreement with previous results on transplanted bivalves, that were good accumulators of the metals Cu and Cd and, in the case of the clam *C.fluminea* specially Pb (Reinfelder et al., 1997; Gunther et al., 1999).

Table 3. Descriptors of the fitted line for the results of concentrations in clams and in sediments. R^2 for uncorrected sediment concentrations and $R^{2'}$ when the concentrations in sediments are corrected for the organic matter content of sediments.

Sample	Slope	R^2	$R^{2'}$
As	-0.009	0.0914	0.6385
Cu	0.0216	0.326	0.688
Zn	0.0475	0.777	0.81
Cd	0.1212	0.985	0.9623
Hg	0.0044	0.5832	0.5338
Pb	0.011	0.794	0.7863

Both biological and geochechical characteristics play important roles in metal bioavailability to the clam *R.philippinarum* (Fan and Wang, 2001). Among others, organic carbon and acid volatile sulfide are found to be critical in affecting metal bioavailability from contaminated sediment (Ankley et al., 1996; Chapman et al., 1998). In this study we only considered the TOM content, but the correction of the sediment concentrations for this factor resulted in a considerable increase in the correlation between the concentrations of Cu in sediments and the corresponding concentrations in clams (Table 3). For this metal, this factor may be determining in the bioavailability and the corrected data are better indicators of the bioaccumulation to clams. These results have been previously found for the deposit-feeder *Arenicola marina* exposed to these same sediments (Casado-Martínez et al., submitted), although for this last organisms this factor also explained some results of Pb and Zn.

The calculation of the accumulation factors (AFs) as the ration of the concentration of a contaminant in bivalves after exposure to the pre-exposure concentrations has been used to assess the proportion of sediment-bound contaminant that is bioavailable. This ratio was very variable among sediments and for different contaminants in a same sediment (Table 4). The highest AFs were reported for Hg, while the lowest values were calculated for Ni, Cu and Zn.

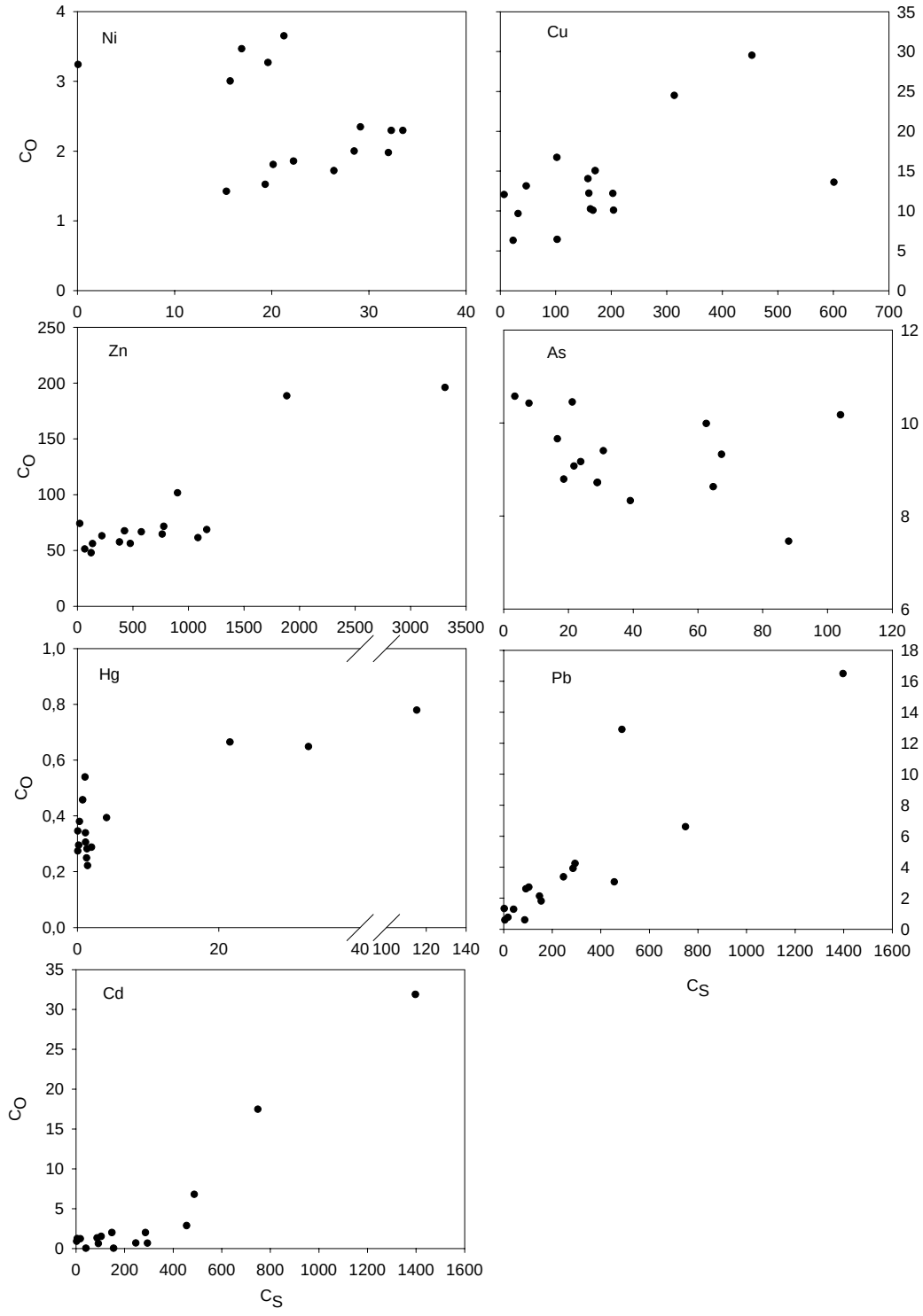

Fig. 1. Plot of concetrations in sediments and in organisms. All concentrations expressed as mg L^{-1} d.w.

Table 4. Calculated AFs for each compound and each sample.

Sample	Ni	Cu	Zn	As	Cd	Hg	Pb
CA1	54.010	1.728	3.485	3.092	0.252	1.41	5.475
CA2	0.090	0.060	0.152	0.306	0.178	1.52	0.145
CA3	0.205	0.281	0.415	0.582	0.161	1.95	1.357
CA4	0.172	0.301	0.782	1.335	0.174	1.78	6.921
B2	0.093	0.078	0.100	0.133	0.119	1.48	0.020
B3	0.014	0.065	0.059	0.085	0.130	2.02	0.007
B4	0.079	0.088	0.113	0.160	0.129	2.77	0.031
C2	0.062	0.049	0.092	0.098	0.200	2.35	0.155
C3	0.191	0.274	0.392	0.418	5.354	1.14	1.639
C4	0.069	0.089	0.057	0.213	0.419	3.33	0.504
BI1	0.070	0.060	0.085	0.302	0.354	4.01	0.193
BI2	0.167	0.063	0.116	0.386	7.826	3.42	0.207
BI3	54.010	1.728	3.485	3.092	0.252	2.42	5.475
PA1	0.090	0.060	0.152	0.306	0.178	2.06	0.145
PA2	0.205	0.281	0.415	0.582	0.161	1.59	1.357
PA3	0.172	0.301	0.782	1.335	0.174	2.64	6.921
H1*	0.081	0.076	0.159	0.493	0.393	1.57	0.303
H2*	0.083	0.164	0.287	0.474	0.470	1.75	0.266
H3*	0.071	0.023	0.059	0.301	0.106	1.45	0.095
C1*	0.065	0.063	0.118	0.139	0.149	1.28	0.618

*Concentrations in clams measure after a shorter exposure than for the rest of sediment.

As recommended by several environmental agencies, the bioaccumulation potential from dredged material is assessed by comparing tissue concentrations after exposure to test sediments in relation to tissue concentrations after exposure to a control or reference sediment (PIANC, 2006; Chapman and Anderson, 2005; Porebski et al., 1999). The accumulation of certain contaminants to much higher concentrations than that found in water, the easy handling and maintenance, the tolerance to contaminated environments and the inability to metabolize contaminants suppose important advantages in using bioaccumulation in bivalves for identifying metal bioavailability. Nonetheless, polychaetes are also recommended in several countries performing these tests for dredged material characterisation (PIANC, 2006). The dredged sediments being assessed were previously characterised for the concentrations of metals in individuals of the species *Arenicola marina*

(Casado-Martínez et al., submitted), and despite the differences in the route of exposure of these organisms to sediment-bound contaminants, both offer similar trends for different metals measured. The general trend in bioaccumulation depicted similar trends for both organisms, although it seems that the concentrations on clams are less variable at intermediate range of concentration. Even if these results may indicate that it is more difficult to relate the bioaccumulation of metals in *A.marina* due to a higher number of factors that contribute to the final tissue residues, the variability could be also caused by the longer exposure period for clams and especially by the different exposure to sediment-bound contaminants. Nevertheless, the use of one or another species should be based on the questions being addressed and especially taking account of the exposure routes and the organisms under risk (Ruus et al., 2005).

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Capítulo 6.

Optimización de un método integrado para la evaluación de la calidad ambiental de materiales de dragado

A lo largo de esta memoria se han discutido algunas de las metodologías utilizadas para la caracterización de la calidad ambiental de materiales de dragado y, en general, sedimentos contaminados. En este capítulo se describe la caracterización de la calidad ambiental de materiales de dragado pero desde un punto de vista integrado. Esta caracterización se realizará mediante la aplicación de un método escalonado.

En el primero de los trabajos que se incluyen en este capítulo, trabajo XVI, se realiza una revisión de las guías químicas y ecotoxicológicas utilizadas para la caracterización y gestión de materiales de dragado. Este trabajo, realizado dentro de las actividades de la Red Española de Sedimentos y en colaboración con la Universidad de Cantabria y el centro AZTI, se muestra la gran variabilidad de los criterios químicos utilizados en la caracterización de materiales de dragado, que se mueven en unos rangos muy amplios de concentraciones. Aunque todas ellas gozan de numerosas ventajas, se recomienda su uso sólo en el contexto para el que fueron desarrolladas. Aunque también se están utilizando guías ecotoxicológicas, su uso no es tan extendido y, al contrario que para las químicas, se recomiendan raramente valores numéricos para la interpretación de este tipo de resultados.

Recientemente han aparecido numerosos cuadros de evaluación escalonados para sedimentos contaminados y/o materiales de dragado, como el publicado por Chapman y Anderson (2005) para sedimentos contaminados o por Stronkhorst (2003). Asimismo, también es de reciente publicación el documento de referencia para el desarrollo de este tipo de cuadros de

evaluación de la calidad ambiental de la PIANC (2006). En estos documentos se puede encontrar cómo caracterizar de forma integrada sedimentos contaminados y/o materiales de dragado, describiendo el tipo de información necesaria, las guías de calidad para la interpretación de los resultados, así como el proceso de integración de las distintas líneas de evidencia para la clasificación definitiva de los materiales. En todos estos cuadros de evaluación y gestión siguen lo que se ha denominado un método escalonado, diseñado para pasar de evaluaciones sencillas que utilizan información ya disponible, a evaluaciones más costosas que ofrecen una información más detallada (Babut et al., 2003). Según ambos documentos, el cuadro de gestión pretende ser objetivo, transparente, riguroso y de fácil consecución. Entre las principales ventajas de este tipo de caracterización es que minimiza la incertidumbre a la hora de seleccionar la opción de gestión más adecuada y, en definitiva, se minimiza el riesgo ambiental de las actividades de dragado. Por el contrario, la utilización de más de una línea de información puede aumentar considerablemente el tiempo y los costes necesarios para determinar si el vertido al mar puede realizarse libremente o de forma controlada o bien se necesita algún tipo de medida correctora. En cualquier caso, el uso de un método escalonado asegura la minimización de gastos, aunque el resultado va a depender en todos los casos de las metodologías utilizadas para alimentar las distintas líneas de información.

Según el documento de referencia, el cuadro de gestión debe incluir medidas de tipo físico, químico y biológico, y, aunque se dan algunas recomendaciones sobre las técnicas de mayor estandarización, no se recomienda una batería de técnicas universal. Por el contrario, se tiende a establecer baterías de ensayos a nivel nacional o regional (Pérez, 2006; Stronkhorst, 2003), su uso rutinario necesita la validación previa. En el trabajo XVII de este capítulo se propone un método escalonado para la mejora del método actual de caracterización y gestión de dragados portuarios en España de acuerdo a las nuevas recomendaciones internacionales. A través de la integración de los resultados presentados en los capítulos II, III, IV y V, se propone una batería de ensayos biológicos para su uso rutinario dentro del cuadro de caracterización y gestión de materiales de dragado con una contaminación intermedia, es decir, materiales de Categoría II. En este trabajo se presentan los resultados más significativos obtenidos durante el proceso de validación de estos ensayos biológicos y se dan las guías necesarias para su desarrollo en el laboratorio, incluyendo las especies y los protocolos de ensayo, y los factores que pueden provocar confusión a la hora de interpretar los resultados dentro del cuadro de gestión propuesto. Finalmente, y de acuerdo con los resultados disponibles, se recomiendan las guías de calidad para la clasificación de los materiales según la respuesta biológica encontrada.

El cuadro de caracterización y gestión resultante contempla una primera fase inicial donde se recoge toda la información disponible sobre la zona a dragar, la zona de vertido propuesta, y sobre el proyecto en sí. De acuerdo a esta información se seleccionará la estrategia de muestro y las estaciones donde se llevará a cabo un estudio más en profundidad, establecido en las siguientes fases del cuadro escalonado. Siguiendo las RRGMMD, el proceso de caracterización y gestión está basado en los denominados NNAA, estableciendo tres categorías de materiales según el grado de contaminación presente. Los materiales de dragado pertenecientes a la Categoría I podrían ser considerados aptos para su vertido al mar previa evaluación de posibles efectos adversos como los producidos por el aumento de la turbidez en la columna de agua o por otros efectos de tipo mecánico en los organismos bentónicos. De forma similar, los materiales de Categoría III se considerarían no aptos para su vertido libre ya que, de acuerdo con las altas concentraciones de contaminantes presentes, los efectos adversos en los organismos de las zonas afectadas parecen muy probables.

Para los materiales con concentraciones intermedias de contaminantes, es decir, materiales de Categoría II, se recomienda la aplicación de una batería de ensayos que incluye el ensayo sobre una población de bacterias de la especie *Vibrio fischeri*, estandarizado y comercializado como Microtox® siguiendo el protocolo para la fase sólida SPT, el ensayo con embriones del erizo de mar *Paracentrotus lividus* para evaluar la toxicidad de las fases lixiviadas de los sedimentos, y el ensayo de supervivencia con anfípodos de la especie *Corophium volutator*, o bien con organismos de las especies de anfípodos de importancia regional *Ampelisca brevicornis*, *Corophium multisetosum, Corophium urdaibaiensis* o *Mycrodeutopus gryllotalpa.*

Como criterios de toxicidad para estos ensayos se considera un valor numérico para el Microtox® similar al criterio canadiense establecido en 1000 mg L^{-1} y referido al peso seco de la muestra. Para los ensayos de desarrollo embrionario con erizos y mortalidad de anfípodos, se considera la diferencia estadística respecto a un control. En el caso de los ensayos sobre la fase líquida este control negativo será el agua utilizada para la obtención de los extractos ensayados mientras que para ensayos sobre la fase sólida se recomienda un sedimento de referencia. La identificación de toxicidad en cualquiera de los ensayos realizados supondrá la clasificación de los materiales como materiales de Categoría III y se procederá según lo establecido para este tipo de materiales. En caso de que los materiales no presenten toxicidad en ningún caso, los materiales se gestionarán según lo establecido para materiales de Categoría I.

Siguiendo las tendencias actuales en gestión de este tipo de materiales, el método escalonado propuesto reconoce la singularidad en el comportamiento y distribución ambiental de los contaminantes de tipo orgánico identificada previamente en esta memoria. Aunque las

RRGMMD separaban dos grupos de contaminantes, uno de estudio necesario y un segundo de interés en el caso de excederse ciertos NNAA, parece más recomendable separar aquellos compuestos que pueden sufrir procesos de bioacumulación y biomagnificación a lo largo de la cadena trófica de aquellos que no. Así, si los materiales de dragado presentan contaminación por este tipo de contaminantes -PCBs, PAHs, compuestos orgánicos del Hg, u otros- se recomienda el estudio de la bioacumulación potencial de estos compuestos. Siguiendo un método escalonado, en primer lugar se pueden realizar estimaciones mediante modelos teóricos utilizados en otros países, como el TBP, seguido de ensayos que consideran las concentraciones en organismos bentónicos tras la exposición a los materiales de dragado en el laboratorio. Tras los primeros resultados, se recomienda el uso de poliquetos de la especie *Arenicola marina* y/o bivalvos de la especie *Ruditapes philippinarum*. Aunque existen muchas dificultades para dar un significado ecológico a los resultados de este tipo de ensayos, la comparación de las concentraciones medidas en los organismos pueden ser interpretadas fácilmente por comparación con concentraciones medidas en un sistema de referencia, como pueden ser en organismos procedentes de la zona de vertido o bien en organismos expuestos en el laboratorio a un sedimento considerado para tal fin.

Para cerrar este capítulo, el trabajo XVIII presenta los resultados de la aplicación de esta metodología integrada para un caso de estudio con puertos afectados por contaminación de tipo metálica: el puerto de Huelva y el puerto de Cartagena. Este trabajo fue presentado en el Congreso mundial de la asociación PIANC celebrado en Lisboa en mayo de 2006 y fue reconocido con el Premio de la Asociación Internacional de Empresas Dragadoras (Internacional Association of Dredging Companies –IADC-), otorgado a la contribución más significativa a la literatura relacionada con las actividades de dragado presentada por un joven profesional, y reconocido con la publicación en la revista de difusión "Terra et Aqua".

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Chemical and ecotoxicological guidelines for managing disposal of dredged material

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Different conventions around the world have produced guidelines for the disposal of dredged material (e.g., London Convention 1972 (LC) (www.Londonconvention.org); Oslo/Paris Convention (OSPAR) (www.ospar.org); and, the Helsinki and Barcelona Conventions). They suggest the use of different methodologies from physico-chemical to biological approaches to the management of different routes of disposal or uses of the dredged material.

Most of these conventions propose methods based on a 'weight of evidence' (WOE) approach. It consists from the beginning of a simple screening approach and progresses to more detailed assessments in cases where any initial management concerns cannot be discounted. Initial assessment considers factors relating to the dredged material, such as the particle-size characteristics of the dredged material, local and historic information, and its environment (e.g., surrounding industries, pollution sources, and collectors). If this information is insufficient to provide answers for the management proposed for it, then further data must be collected that involves chemical characterization of the dredged material. In some countries, these primary assessment processes also incorporate biological screening tests.

If management decisions regarding sediment quality cannot be made from initial and primary assessments, direct measurements of toxicity and/or bio-accumulation and/or other case-specific studies may be required, until it is judged that sufficient information is available for a decision to be made about the disposal or use of the dredged material.

We aim to review the different methodologies used for characterizing dredged material with an special focus on Spain, describing recent initiatives that include revision of some of the most common tests used to assess sediment quality and potential guidelines for the management of the disposal of dredged material.

Keywords: Contamination; Sediment-quality guidelines (SQG); Tier testing; Toxicity tests; Weight of evidence

1. Introduction

Dredging activities are associated with some economic, social and environmental necessities (e.g., navigation facilities and beneficial uses (e.g., beach restoration, agriculture, land fill, and construction) that remove highly contaminated sediments to clean up an area, and mining). Sediments therefore need protection and conservation.

Depending on their physico-chemical properties as well as geochemical and hydrodynamic conditions, sediments can act as a source of, or as sink for, contaminants, thus preserving the history of mankind.

Dredging operations imply the removal of sediments, and their transport and relocation. During these activities, sediments can enhance their capacity to mobilize contaminants, which means

there is an environmental impact that should be taken into account. Furthermore, most of the dredged sediments arise from ports, where, normally, sediments are heavily contaminated because of the anthropogenic input from the surroundings and to the high rates of sediment accumulation in port basins. However, for many years, disposal of this contaminated material at sea was considered best practice, without any concern about its environmental impact.

Since 1950, the first signs of marine contamination started to appear in Europe and to create a certain concern among the scientific community. But, in 1972, at the Human Rights Conference in Stockholm, the obligation of all countries to protect the marine environment was declared. After this historical date, several International Conventions for the Protection of Marine Environment were elaborated for different marine regions (e.g., Mediterranean Sea; Gulf Area; Central and Western Africa; Eastern Africa; South Pacific; Red Sea; the Gulf of Aden; Caribbean Region; North-eastern Atlantic; and, Baltic Sea). In addition, LC and the International Convention for the Prevention of Pollution from ships (MARPOL 73/78) were also held, both relating to the spillages and contamination from tankers at sea. In 1986, The Specific Guidelines for Dredged Material were approved and then summarized and updated in the 1996 Protocol [1].

Spain has been party to LC, MARPOL, OSPAR (Northeastern Atlantic) and Barcelona (Mediterranean Sea) since 1974 and 1976, respectively. However, at that time in Spain, there were no regulations to characterize the dredged material and to control its disposal. The first document regarding the characterization and control of dredged material was published in 1994, *Recommendations for the management of dredged material in ports of Spain,* RMDM [2]. Although these recommendations have no legislative force, they have been applied routinely to characterize the dredged sediments in the Spanish ports.

2. Chemical guidelines

The Spanish RMDM and other proposals for initial tier testing for characterizing dredged material by different regulatory agencies (e.g., US EPA, Environment Canada, Environment Australia, and Dutch agencies) are mainly based on a chemical approach using chemical guidelines (Table 1). However, each country has developed particular guidelines in order to manage the dredged material, with different competence in practice (e.g., in Spain two action levels were proposed and three concentration categories were defined). The contaminants are measured in fine sediments (< 63 μm). These actions levels for management of Spanish dredged material are shown in Table 1. Thus, concentrations below action level 1 (or target level) mean that disposal is permitted taking into account the physical effects associated with this process. This material is considered category I.

Dredged materials that show concentration of contaminants above action level 1, but below action level 2, are considered category II and indicate moderate contamination. This material would require further study before disposal could be permitted.

Dredged materials above action level 2 (limit or intervention level) belong to category III and must be isolated into containers or into a contained area.

For particularly contaminated materials that show concentrations of contaminants higher than 8 times the value of action level 2, special isolated techniques are requested.

Category I: $C < AL1$, $C =$ concentration.
Category II: $AL1 < C < AL2$, $AL =$ action level.
Category IIIa: $C > AL2$.
Category IIIb: $C > 8^{*}AL2$.

In the disposal area, RMDM requires study of bottom-sediment typology and biological reconnaissance of the bottom. The latter can include determination of biomass per unit of surface area, diversity, and visual inspection.

3. Deriving Sediment Quality Guidelines (SQG)

Table 2 shows the basis, formulations, advantages and limitations of several SQG approaches [3,4]. In Table 2, three different approaches are gathered (empirically based, theoretically based and consensus). It sets out the advantages (+) and disadvantages (−) of each method. Because of the controversial issues associated with SQG, the use of numerical guidelines based on different or tiered approaches is recommended [5,6]. Theoretical and empirical methods have attempted to describe ionic and non-ionic toxicity in sediments, but the connection between toxicity and chemistry is weak [7,8].

The Spanish SQG are based on geochemical considerations instead of being related to toxicological or biological effects. In this sense, general guidelines are not often useful for the management of dredged material and they should be site specific [9,10]. Thus, they should be recommended only for use in specific areas for which they were derived using multiple lines of evidence under WOE considerations.

There are cases in which no specific toxicity data for the relevant constituents and bioassay are available or in which further biological assessments are not recommended because of cost. Then, it might be possible to rely on SQG previously derived for the area from the literature. SQG are either based on a theoretical, mechanistic approach or on empirically derived numeric values (Table 2).

In general, SQG will properly identify those contaminants that are of no concern. In summary, SQG are only

Table 1. SQG proposed by different agencies and authors for marine sediments

Parameter	Other values	Reference CEDEX [2]		Dutch SQG[a] [22]				Long et al. [13]		McDonald et al. [16]		Riba et al. [24]	
		AL1	AL2	Target value	Threshold value	Test value	Action value	ERL[b]	ERM[c]	TEL[d]	PEL[e]	V1	V2
As	–	80	200	29	55	55	55	8.2	70	7.2	41.6	27.4	213
Cd	–	1	5	0.8	2	7.5	12	1.2	9.6	0.68	4.21	0.51	0.96
Cr	–	200	1000	100	380	380	380	81	370	52	160	–	–
Cu	–	100	400	35	35	90	190	34	270	19	108	209	979
Hg	–	0.6	3	0.3	0.5	1.6	10	0.15	0.71	0.13	0.70	0.54	1.47
Ni	–	100	400	35	35	45	21,044	20.9	51.6	15.9	42.8	–	–
Pb	–	120	600	85	530	530	530	46.7	218	30.2	112.2	260	270
Zn	–	500	3000	140	480	720	720	150	410	124	271	513	1310
$\sum_7$-PCB	–	30	100	–	–	0.2	1	22.7	180	22	180	54	254
$\sum_{10}$-PAHs	–	–	–	1	1	10	40	–	–	–	–	–	–
$\sum_{13}$-PAHs	10[f]	–	–	–	–	–	–	4000[g]	45,000[g]	624	15,200	–	–

All values are expressed as mg/kg except $\sum_7$-PCB and $\sum$-PAH expressed as μg/kg.

[a] Dutch SQG [22]: [Concentration] < target value, the material is class 0 (non-polluted). Target < [Concentration] < threshold, material Class 1 (slightly polluted). Threshold value < [Concentration] < Test value, material class 2 (moderately polluted). Test < [Concentration] < action, material class 3 (polluted). [Concentration] > action value, material class 4 (heavily polluted).

[b] ERL (effects range-low): (percentile 10th) represents values below which biological effects are expected to be rare.

[c] ERM (effects range-median): (percentile 50th) represents values above which biological effects are expected frequently.

[d] TEL (threshold effects level): from this value adverse effects can be frequent.

[e] PEL (probable effects level): level above which adverse effects are frequently expected (geometric mean of the 50% of impacted toxic samples and the 85% of the non-impacted samples).

[f] Extreme effect concentration (EEC) value from the consensus approach SQG [20].

[g] It refers to the total PAHs (high and low molecular weight).

Table 2. Main characteristics of the different approaches used to derive SQG

SQG Designed specifically to support strategic actions to assess, limit or prevent additional contamination	
Empirically based approaches: Based on measured chemical concentrations and corresponding observed biological effects (e.g., growth, mortality, and reproduction). Correlative approach SQG	
•Species screening level concentration (SSLC) [12]	•Probable effect levels (PELs) and threshold effect levels (TEL) [16]
•Effect ranges low and medium (ERL and ERM) [13,14]. ERM Quotient [14]	•Summed PAH model ($\sum$ PAH) [17]
•Low and high apparent effect threshold (LAET) and (HAET) [15]	•Ecotoxicological assessment criteria (EAC) [21] •Triad and sediment bioassay [9–11,35]
(+) Based on sound empirical method that utilize large existing databases of correlative effects	(−) Do not show causal effects. False negatives and false positives
(+) May be expanded to be applied to a regulatory framework	(−) Do not resolve the issues of Bioavailability (based on d.w. concentrations)
Theoretically based approaches: Based on an understanding of the partitioning of chemicals in the sediments and the toxicity of the dissolved fraction in the sediment interstitial water. Equilibrium approach SQG	
$ESG_{OC} = K_{OC}$ (WQCFCV) [4] ESG_{OC}: Equilibrium sediment guidelines based on organic carbon; K_{OC}: Organic carbon partition coefficient; WQCFCV: water quality criteria final chronic value	EqP [18,19]
(+) Based on fundamental toxicological principles that causally link the bioavailable concentration of contaminants in sediments to specific responses of benthic organisms.	(−) Not intended to protect against additive, synergistic or antagonistic effects of other contaminants
(+) Used to derive sediment guidelines for metal and PAH mixtures	(−) Only developed for some non-ionic compounds and five divalent metals.
***Consensus approach* [20]:** Combines sediment guidelines from correlative and EqP approaches to create a consensus threshold of effects concentration (TEC), medium effects concentration (MEC), probable effect concentration (PEC) and extreme effect concentration (EEC)	
(+) Provide a unifying synthesis of other SQGs	(−) Additional field data are needed to validate
(+) Reflect causal rather than correlative effects. Account for mixtures.	(−) Bioavailability overestimation of inert fractions

used as a first tier in the whole tier-testing schema recommended for the assessment of the hazardousness of sediments [11] and dredged material, and they should not be used alone for regulatory proposes. This is supported by results associated with different SQG and actions levels proposed by different regulatory agencies, convention and authors, as shown in Fig. 1. Fig. 1 shows the range of variation for empirical, theoretical and consensus-based SQG as effect ranges low and medium (ERL and ERM), apparent effect threshold (AET), probable effect levels (PELs) and threshold effect levels (TELs), summed PAH model ($\sum$PAH), ecotoxicological assessment criteria (EAC) used by OSPAR, threshold of effects concentration (TEC), medium effects concentration (MEC), probable effect concentration (PEC), [12–21] as well as the range of variation of different action levels or management guidelines for dredged material [1,22–24]. SQG defined for individual chemicals and groups of chemicals differ by more than one order of magnitude and show high variability for a same compound. Also, differences among actions levels for each country are greater than those among SQG that show a narrow area of uncertainty. It is necessary to point out that total PAHs show a four orders of magnitude difference that is associated with differences in the definition of the number of PAHs to be included. Although equivalence between different SQG, such as the Dutch values, defined as $\sum_{10}$-PAHs, and consensus SQG, defined as $\sum_{13}$-PAHs, has been reported [25], there is no consensus around the world to show the same PAH compounds as this definition, and this makes the greatest difference for these compounds, as shown in Fig. 1. An emerging contaminant (e.g., tributyltin (TBT)) also appears to have a big difference in action levels but with narrow variation in its respective SQG values.

The decision-making framework for the disposal of dredged material in the marine environment, comprising the hazard assessment of sediment, the risk assessment of the receiving environment, the use of chemical SQG (in rectangles, Fig. 1), together with social, political and economical aspects, results in action levels or management guidelines for dredged material (in circles, Fig. 1).

Sediment chemistry is the main method by which assessment of the dredged material is made, although some European countries (e.g., Belgium, Germany and Spain) are introducing ecotoxicological measures; in this sense, these values should be noted if they will be used for management as part of a tier-testing approach; they should never be used alone. The most common approach involves two action levels, although Portugal uses a five-category system for assessing dredged material, and a few countries operate a case-by-case system.

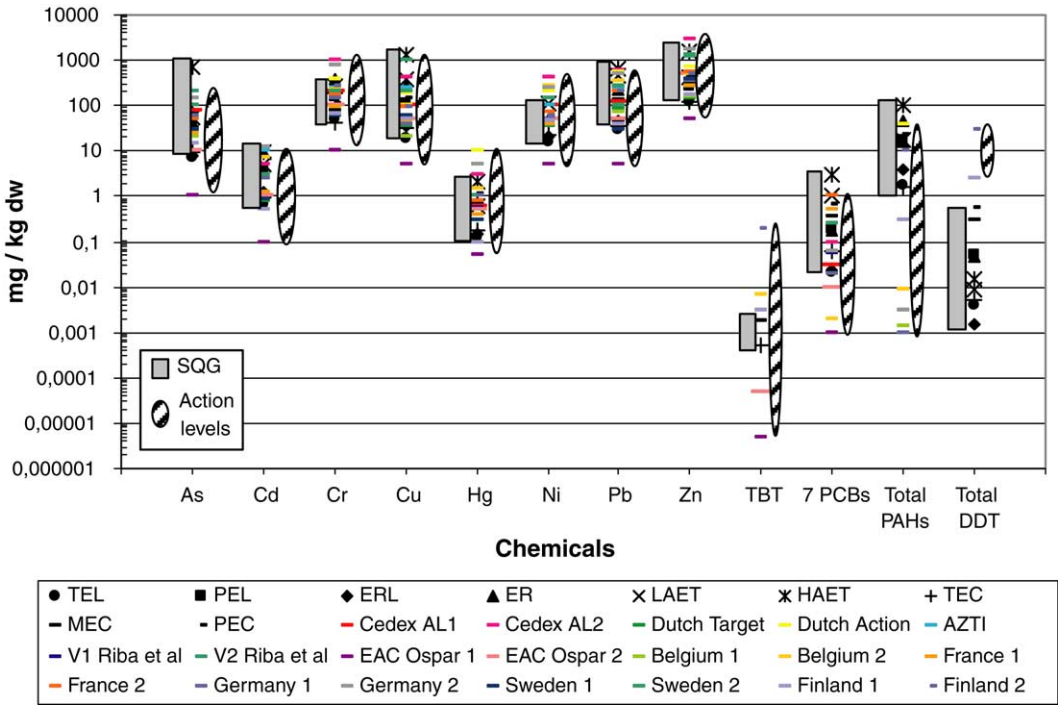

Figure 1. Summarized representation of benchmark guidelines (SQG and action levels) obtained from different regulatory agencies, conventions and authors cited in this caption. TEL, PEL, ERL and ERM [13,14]; LAET and HAET [15]; TEC, MEC and PEC [20]; EAC OSPAR [21]; Dutch values [22]; AZTI values [23]; Riba values [24]; CEDEX values [2]; and, Belgium, Finland, France, Germany and Sweden values [36].

Fig. 2 shows the individual and groups of priority substances in the field of the Water Framework Directive (WFD) and other substances, which are adopt by European countries as criteria for assessing the disposal of dredged material. At the inner circles are the countries adopting different substances in their legislation. The majority of countries take into account the total concentration of arsenic and metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn), but a more limited number of countries take into account their speciation; in fact, only Belgium and Finland have established that tributyltin (TBT) in dredged material has an action level. The number of individual PAHs and Total PAHs differs from country to country and do not coincide with the priority substances in the WFD. Emerging contaminants (e.g., phthalates, brominated flame retardants (BFRs), nonylphenols, octylphenols and pesticides, which exhibit potential harmful effect in the environment [26] and are defined as priority substances in the EWF) are hardly included in the legal frameworks of European countries as criteria for dredged material (Fig. 2).

The action levels for dredged materials in European countries are heterogeneous, with differences in the numerical values (Fig. 1) and in the chemicals taken into account (Fig. 2). In addition, these values are based on the use of fractions with different grain sizes (<2 mm, <63 μm or <20 μm), different standards for organic matter (normally 10% of TOC), determination of metals by total or partial digestion, and determination of organics using different extraction solvents and methodologies.

4. Should further assessments include biological guidelines?

The complex matrix of dredged material places limitations on the use of chemical analytical methods alone for estimating the bioavailability and the toxicity of contaminants present. The impact of contaminants on sediment biota depends on their bioavailability (i.e. the extent to which they can be taken up by sediment organisms), so assessment of the risks of sediment contaminants should include consideration of their bioavailability.

However, it is difficult to estimate the bioavailable fraction in sediments. Several approaches have been tried with various levels of success (e.g., extraction with weak acids, sequential selective extraction methods or the acid volatile sulphide approaches) [11]. Recently, new approaches have been developed based on diffusive sampling within sediments. As far as metals are concerned, no single method seems sufficiently developed to reach any firm conclusions. More experience and information seems to be required. For organic, hydrophobic contaminants, such as PCBs and PAHs, the

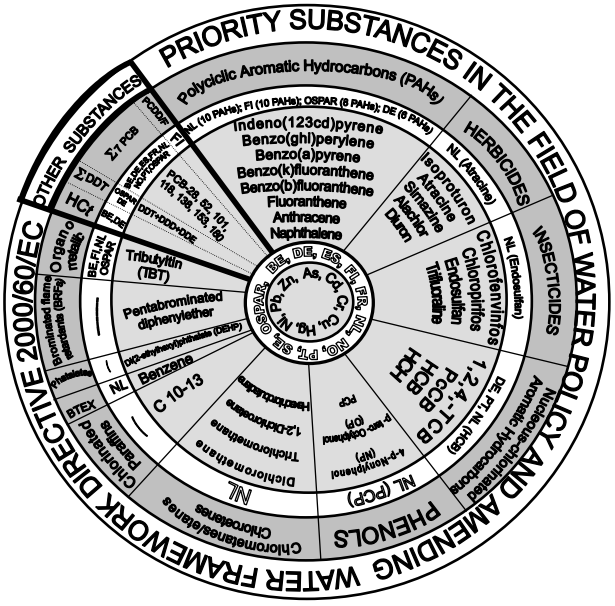

Figure 2. Priority substances in the Water Framework Directive and other substances used to define the action level for assessment of disposal of dredged material in different countries: Belgium (BE), Germany (DE), Spain (ES), Finland (FI), France (FR), Norway (NO), Portugal (PT) and Sweden (SE). Chemical abbreviations: BTEX, benzene, toluene, ethylbenzene, xylene; C10–13, C_{10-13} chloroalkanes; HCB, hexachlorobenzene; HCH, γ-hexachlorocyclohexane; HCt, total hydrocarbons; PCB, polychlorinated biphenyl; PcCB, pentachlorobenzene; PCDD/F, polychlorinated dibenzo-*p*-dioxin/dibenzofuran; PCP, pentachlorophenol; 1,2,4-TCB, 1,2,4-trichlorobenzene.

diffusive sampling approach is more adequate, but not easy.

As a result, biological testing is becoming widely accepted for characterizing the chemical hazards in dredged material, and for providing information to support the process of evaluating the impact of the dredged material. By exposing relevant organisms under controlled conditions to samples of the material to be dredged and then measuring toxicological effects (e.g., mortality or reduced growth) and/or the bio-accumulation of contaminants into tissues, estimates can be made of the chemical hazards present.

These methods can be of considerable value in assessing the benefits of using the dredged material and the impact of disposal in the aquatic environment.

5. Deriving biological guidelines from bioassays

Sediment bioassays are intended to determine the potential chemical toxicity and/or bioaccumulation of a dredged material as distinct from its physical characteristics (e.g., grain size). Control and reference sediments should be selected to minimize any artificial effects of differences in grain size and other natural variables.

Organisms used in sediment tests will be affected by contaminants through different routes by (e.g., exposing them directly to contact with sediments or to sediment suspended in the water column, incorporating them as part of food or other particles, and producing bioaccumulation and trophic transfer within a food chain). Different tests will need to be applied to address assessment questions associated with these routes. Sediment-toxicity tests use different pathways of exposure through whole sediment or elutriates (even interstitial waters) and measure endpoints of effects (e.g., mortality, or growth reduction). Bioaccumulation tests are conducted to establish the incorporation of the chemical into the organism and through it within the food chain. They offer certain information about exposure and human risk. These bioassays for different routes and objectives are included in the different guidelines for characterizing dredged material around the world; Table 3 shows some examples.

The wise way to use sediment bioassays in the management of dredged material is to design a battery of tests that will give information on acute effects (e.g., survival of amphipods), sub-lethal and/or chronic effects (e.g., growth, and reproduction) and bioaccumulation, as appropriate. The battery should also be representative

Table 3. Examples of sediment bioassays recommended around the world by different agencies or governmental bodies to be used as part of a tier-testing approach to the management of dredged material

Type	Test	Country
Screening	Microtox (STP)	The Netherlands Canada Australia Spain
Solid phase	Amphipods	The Netherlands Canada USA UK Australia Spain
	Benthic algae viability	Australia
Liquid phase	Sea urchin embryo development	Canada Spain USA
	Sea urchin fertilization and larval development	Australia USA
	Bivalve larval development	Australia
	Tiger prawn survival (post-larvae)	Australia
	Algal growth inhibition test	Australia
Bioaccumulation	Bivalves	Canada USA
	Polichaetae	USA

of the predominant routes of exposure, including sediment and possibly water (extracts, elutriates) or tissues (to assess bioaccumulation) in the area of study or the country of application.

Biological guidelines define some values of endpoints included in different sediment bioassays. The aim of these values will be to determine if a sample of dredged material is associated with hazard or not compared to a reference or control sediment. Based on these values, the material can be managed and a decision made whether it is beneficial or not to freely dump it.

To be part of a tier-testing approach for regulatory proposes, the bioassays require the involvement of the regulatory agencies that should address the uncertainties associated with all analytical tools used in the evaluative process, including biological tests. In this sense, Table 4 sets out some basic characteristics that a bioassay should have before being included in recommendations for dredged materials.

6. Linking sediment and biological guidelines

We recommend that complementary tools for managing the disposal of dredged materials or other uses should be employed in a tiered fashion.

Most of the recommended tier-testing approaches from all over the world involve initial sediment chemistry or similar ''screening tests'' followed by more detailed assessment where the benchmarks for the screening tests are exceeded. For those samples exceeding these values, further tiers are needed and involve acute and/or chronic toxicity tests and specific tests to establish the bioaccumulation of some contaminants of concern associated with the dredged material. Fig. 3 shows one example of a tier-testing approach recommended by the Institute of Fisheries and Food Technology of the Basque Country (AZTI) for managing dredged material in the Basque Ports that the Basque Government supports.

Initial testing involves full documentation of the area (industrial history (surrounding industries, contamina-

Table 4. Basic characteristics required for a bioassay to be recommended as part of a tier-testing approach for management of dredged material

Selection of organisms	Ecological relevance in the disposal or concern sites Tolerance of the organism to environmental variables Life cycle Availability of organisms Sensitivity to a high range of contaminants
Selection of endpoints	Address confounding factors Significance Availability to interpret results
Experimental	Meet specific objectives Consistency (experimental conditions perfectly defined) Performance of standards Definition of statistical hypothesis Correct replication Inter-laboratory precision Costs of the whole test (minimizing them) Testing to real world dredged material (not researching in sediments) Availability of standard operation protocols, including interpretive guidance
Ruggedness	Precision in defining features of the tests (e.g., fed animals, and burial of clams): conditions of the sediment and/or water (e.g., grain size, and salinity) Well develop quality assurance/quality control guidance (e.g., mortality acceptable in the negative control of toxicity, range of temperature overlapped, use of correct negative and positive controls, range of temperature, time of storage, and sampling collection and handling)
Ring tests	Correct selection of laboratories. Not only highly scientific but usually laboratories conducting these kinds of methodologies to assess dredged material Correct design including all the potential errors from field collection to the final results for management proposals Correct use of the statistical tool
Validation of the tests	Using different range of sediment conditions Response logical and predictable to a high range of contaminants in dredged material Comparison to other standardized tests
Data-management procedures	The data should be collected and handled during the tests following guidelines to maintain accuracy and quality Validation and confirmation of the accuracy of transcription from laboratory to the final report to ensure credibility of the results

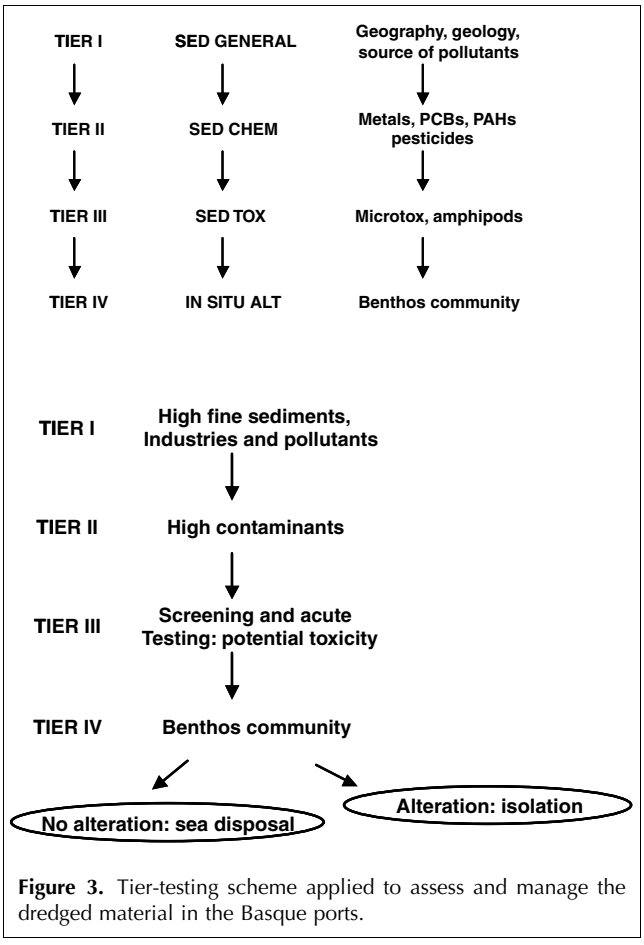

Figure 3. Tier-testing scheme applied to assess and manage the dredged material in the Basque ports.

tion sources, urban nucleus), geology, volume of sediments to be dredged, hydrodynamic); this provides a basis for assuming the presence of contaminants and the affected areas where there may be some impact. On the basis of this information, the strategy of sampling for subsequent tier analysis is defined. The sampling strategy selects the risk areas to be studied in detail. The number of samples is optimized, based on the previous information about the area, although the samples must be representative of the total area to be dredged. Before further testing, the type of material to be dredged is considered. For rocky and sand sediments with gravels and shell debris, further analysis are not necessary. For sediment containing more than 10% of fine material (<63 μm), the next testing step is performed.

Tier II involves analyzing the concentration of contaminants (metals, PCBs, PAHs and pesticides) in the fine fraction and comparing them to action levels proposed by AZTI [23] (see Fig. 1). Also, to provide a reasonable surety that major effects are detected, a Tier III is recommended with a battery of bioassays to determine the toxicity of the material. The selected bioassays are a screening test, Microtox in solid phase with a bioluminiscence bacteria *Vibrio fisheri* [27], and an acute toxicity test with marine amphipods representatives of the Basque estuaries, *Corophium* sp. and *Corophium multisetosum* [28]. Those samples without biological effect can be freely disposed at sea. Samples that present high contaminant concentrations and show biological effects must be isolated into containers or into a contained area.

In a final Tier IV, the level of biological effect is measured under field conditions. Changes in the benthos communities are studied at the area to be dredged and at the disposal site.

Fig. 3 presents a possible framework for integrating both chemical and biological assessment tools into the decision-making process. Tiers allow an assessor to do only as much sampling and analysis as are needed to make a reasonable decision. Moving though the tiers, one moves from a broad to a more focused scope and from general benchmarks to more detailed, directed tests. It should be noted that, although the tiers have been numbered for reference, the process is iterative, with information from one level, informing the questions or considerations at a previous level.

Features of the framework suggested are:

(a) exit at any point where you have enough information – WOE;
(b) each tier increases the WOE that supports the decision on disposal in open water;
(c) each tier also increases the cost, the complexity and the expertise needed;
(d) for secondary assessment tests, choose as many or as few as are needed to enable a decision;
(e) choices should be driven by chemicals of concern, and routes of exposure (conceptual model);
(f) the end result will be a decision to allow open water disposal or to seek other management options; and,
(g) close the loop with an investigation of cause/effect (e.g., TIE) to enable future management and control of sources of contamination.

7. Macrobenthic community structure studies

Benthic community structure studies are recommended in most of the sediment quality assessments conducted under a WOE approach, such as Triad (Fig. 4). Sometimes, results are usefully based on causal linkages between the observed status of the benthic community and the presence of chemical contaminants in the sediment. However, there are drawbacks associated with such studies so that care is required when they are used for assessing dredged material (e.g., navigation channels are subject to physical disturbance, including the dredging operations, and the movement of ship traffic). These disturbances can affect the taxonomic structure of the benthic community and can even produce big differences between the macrobenthic community at the

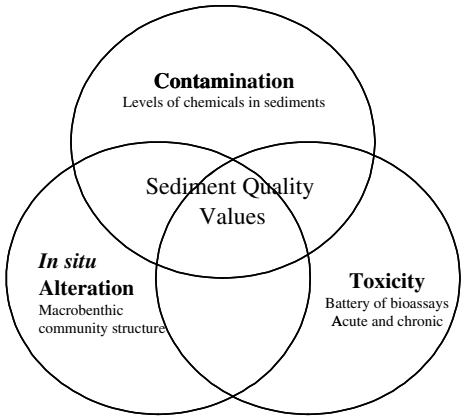

Figure 4. Classical synoptic representation of the WOE approach, named Triad, including in the *in situ* alteration component in the studies of the structure of macrobenthic community (adapted from [9]).

margins and on the slopes of the navigation channel compared to that deep in the channel. Besides, in some areas the total absence of organisms can be related to the extremely stressful conditions resulting from the dredged operations. Thus, information about benthic community structure alone is of limited value in judging the need for special handling or management once the sediment is dredged from the channel. Information about benthic community structure has been used extensively as a part of monitoring programs at dredged material disposal sites; however, effective use of this information also requires distinction to be made between effects caused by the physical disturbance created by a disposal event and effects caused by the presence of chemical contaminants.

In the tier-testing approach recommended for the management of dredged material in ports managed by the Basque Government, the study of change in the benthos community is done at the disposal site. In this sense, some improvements in this study established background levels for the site-specific areas of these ports that were different from those in the rest of Spain (both commercial ports and those under the jurisdiction of the Spanish government). Thus, Borja et al. [29,30] developed the AMBI (AZTI Marine Biotic Index), which has been applied and verified in several geographical areas with respect to the impact of various sources. The AMBI was designed to establish the ecological quality of European coast by investigating the responses of soft-bottom communities to changes in water quality. Hence, the AMBI offers a disturbance classification of a particular site, representing the health of the benthic community. Most of the concepts developed within the AMBI are based upon previous proposals:

(i) the species should be classified into five ecological groups (EG) [31]; and,
(ii) with a scale of values from 0 to 7 [32,33].

The AMBI is very easy to use, having freely available software, including a continuously updated species list, incorporating more than 2700 taxa (www.azti.es/ingles). This index is recommended for tier testing for the management of dredged material in the Basque country, although recommendation for general use in sediment-quality assessment is still under debate. Furthermore, it should be made clear that it is not recommended in the new guidelines for the management of dredged material in the new Spanish recommendations currently under development by CEDEX [34] because of the drawbacks described above and related to the specificity of dredging operations and characterization.

8. Final remarks

The use of SQG based on empirical, theoretical or consensus approaches is not enough to manage the different uses or disposal options for dredged materials. The high variability detected among different action levels, SQG and, in general, in the proposed benchmarks for different regulatory agencies, conventions or authors all over the world determine that they cannot be used alone for management purposes. However, they are a valuable tool when used in a tier-testing approach that includes other methodologies to address the hazards of the dredged materials.

Biological tests are a powerful tool when used for complementary study under a tier-testing approach to address the different options for re-use or disposal of dredged material. However, they are not widely recommended for all the regulatory agencies all over the world, although some improvements are in progress in designing different international protocols and conventions. Furthermore, these complementary new techniques need convenient standardized protocols to be used in deriving helpful biological guidelines for wide application in characterizing dredged materials. The use of other techniques, such as the macrobenthic community structure, could be valid, although important drawbacks should be addressed before it is widely recommended for this purpose, especially when used in highly dynamic and variable ecosystems. A potential use of this tool could be for monitoring at the disposal site instead of characterizing the dredged material. We recommend the development of other tools for this in the next future.

The use of both chemical and ecotoxicological analyses integrated into a tier-testing approach based on a WOE is the most powerful tool for determining the hazards associated with contaminants bound to dredged materials.

Finally, incorporating other different lines of evidence (e.g., chronic toxicity tests, biomarkers,

bioaccumulation, and benthic fluxes) will avoid the drawbacks associated with unidentified chemicals and/or biological responses as well as with uncertainties related to the structure of macrobenthic community studies. These techniques are still under development and they must be standardized if they are to be widely recommended by regulatory agencies.

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Developing Biological Tests and Guidelines as Part of an Integrated Assessment Framework For Dredged Material Characterisation In Spain [1]

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ABSTRACT

This paper summarises events in the development of toxicity bioassays for the evaluation of dredged material intended for open water disposal in a regional context –Spain-. To take advantage of all the literature available, a sound review was performed to elaborate on the current state-of-the-science on sediment toxicity bioassays and identify the most promising tests for regulatory use. For each test, the gaps of knowledge for its application were investigated and were the subject for further testing with dredged materials. Finally the test battery was validated through different interlaboratory exercises, which served to evaluate the tests by multiple laboratories and the needs for continued research and development. After completing different studies, the test battery recommended in Spain would include the Microtox® SP test to screen for toxicity, the amphipod survival test to assess the toxic effects on benthic organisms upon exposure to the whole sediments, and the test with early life stages of the sea urchin *Paracentrotus lividus* to test the elutriates. The biological assessments include bioaccumulation studies as part of the methodology to assess the risks of compounds that can bioaccumulate and biomagnify. A tiered testing approach is proporsed for dredged material characterisation and management in Spain, including both physico-chemical and biological endpoints together with the chemical and ecotoxicological guidelines for the proper deployment of the methodology.

Key words: contamination, bioassays, bioaccumulation

INTRODUCTION

Sediments have served as sinks for many contaminants entering the aquatic environment for centuries and it is not untill the last decades that they are considered a potential source of pollution. This is especially true for navigational dredging as sediment-bound contaminants can be readily available after excavation and remobilization of sediments, posing a risk to the surrounding environment. Although these activities are regulated by several conventions for the

[1] Integrated Environmental Assessment and Management (Enviado)

protection for the protection of the marine environment (i.e. London Convention 1972 -www.londonconvention.org-, the Oslo/Paris Convention -www.ospar.org-), economic factors usually force these dredging activities regardless the contamination status (Peddicord et al., 1997). Spain, as a signing party of these conventions, enacted a permit system for navigational dredging in order to comply with these international conventions, the so called Recommendations for Dredged Material Management (RRGMMD; CEDEX, 1994). This permit system was in agreement with the guidelines enacted by the different conventions and basically followed an action level approach, with different management options depending on the quality and quantity of dredged materials. While the quantity varies for each project, three different categories of materials were distinguished: Category I for materials suitable for open water disposal, Category III for materials that are needed of some techniques to reduce the risks for the surrounding environment, and Category II for those materials that cannot be classified in none of the other two categories.

Traditionally this classification was made acccording to the sediment physico-chemical characteristics, but in the last years biological testing is being incorporated in the context of navigational dredging as part of the new effect-based assessment frameworks (den Besten et al., 2003; LC 1996 Protocol; PIANC, 2006). The chemical measurements may be used on an early step followed by biological measurements (US EPA and USACE, 1991, 1998; Stronkhorst, 2003), while other countries recommend the use of biological endpoints together with the physico-chemical results (den Besten et al., 2003). In the Spanish RRGMMD, the introduction of biological tests was foreseen but was not still recommended because, when these recommendations were enacted, these methodologies were still on an earlier developmental phase (CEDEX, 1994). Because of the economical implications of the regulatory decisions involved, scientists and regulators agree that the research and development of all analytical tools used in dredged material management is justified, as it minimises the uncertainties associated with its application and increases its reliability for decision-making (PIANC, 2006; Peddicord et al., 1997). This paper summarises events in the research performed to develop and validate a sediment toxicity test battery for dredged material characterisation in the context of the Spanish RRGMMD (DelValls et al., 2001; 2003a). Although the results of these events are relevant in a regional context, it describes a general approach that may be used elsewhere to individuate the most suitable methodologies for the regulatory evaluation of dredged materials in agreement with the international recommendations. For this purpose, a modification of the former assessment framework is recommended.

RATIONALE AND ASSESSMENT FRAMEWORK

Generally dredged material assessment and management frameworks are tiered and designed to proceed from single and cost-effective evaluations, which take advantage of available information, to costly assessments that provide more detailed information. This type of assessment framework, which allows to allocate properly limited technical and financial resources (Babut et al., 2003), has been recommended in the last years for contaminated sediments and especifically dredged material worldwide. Several examples have been published recently that can be applied regionally. Chapman and Anderson (2005) proposed a requisite framework for contaminated sediments to determine "when contamination (defined as the condition in which substances are present where they would normally be found or where they occur above natural background levels) becomes pollution (defined as contamination that results in adverse biological effects)". This decision-making framework intended to be objective, transparent, scientifically rigurous, and readily understable, starting with chemical hazard assessment, then adding toxicity tests, followed by environmental evaluations (Chapman and Anderson, 2005; Apitz et al., 2005). The most recent publication by the group of experts on this topic from PIANC (2006) also recommended a generalized assessment and decision-making framework that may provide a "transparent and consistent" process for designing an environmental evaluation, including physical, chemical and biological measurement endpoints to reach, as they stressed, an "informed management decision". Nonetheless, it is worth mentioning that these general frameworks do not specify the methodologies needed to inform the final assessment.

Common to all projects, assessment frameworks begin with an initial assessment tier consisting on the definition of the project scope and the project conceptual model. This allows to identify the contaminants of concern, the resources of concern, the relevant exposure pathways and the available management options. Moreover, this first assessment is used to define the assessment questions that need to be addressed through the following tiers. Apitz et al. (2005) discussed more in detail the need for specific conceptual models, although a standardised conceptual model may be set up in the case of navigational dredging as assessment questions are generally common to all projects (PIANC, 2006). In the Spanish case, the RRGMMD were principally designed to decide whether dredged materials are suitable for open water disposal because, for economical and logistical reasons, the preferred management option for dredged materials is disposal into the same system (Apitz et al., 2005). According to this common approach to all projects, the RRGMMD recommended the use of three types of measurement endpoints to determine the quality of the dredged materials being assessed: physical, chemical and idealy, biological. Similarly they provided with the criteria needed to investigate on the quality

of the dredged materials being assessed. According to the RRGMMD, the dominance of coarse particle sizes may be sufficient to decide that the sediments are absent of contamination, as sediments composed predominantly of coarse/sandy particle sizes have a low probability to carry significant amounts of chemicals due to the relatively small surface area available for sorption of contaminants per unit volume of material (Carpentier et al., 2002; PIANC, 2006).

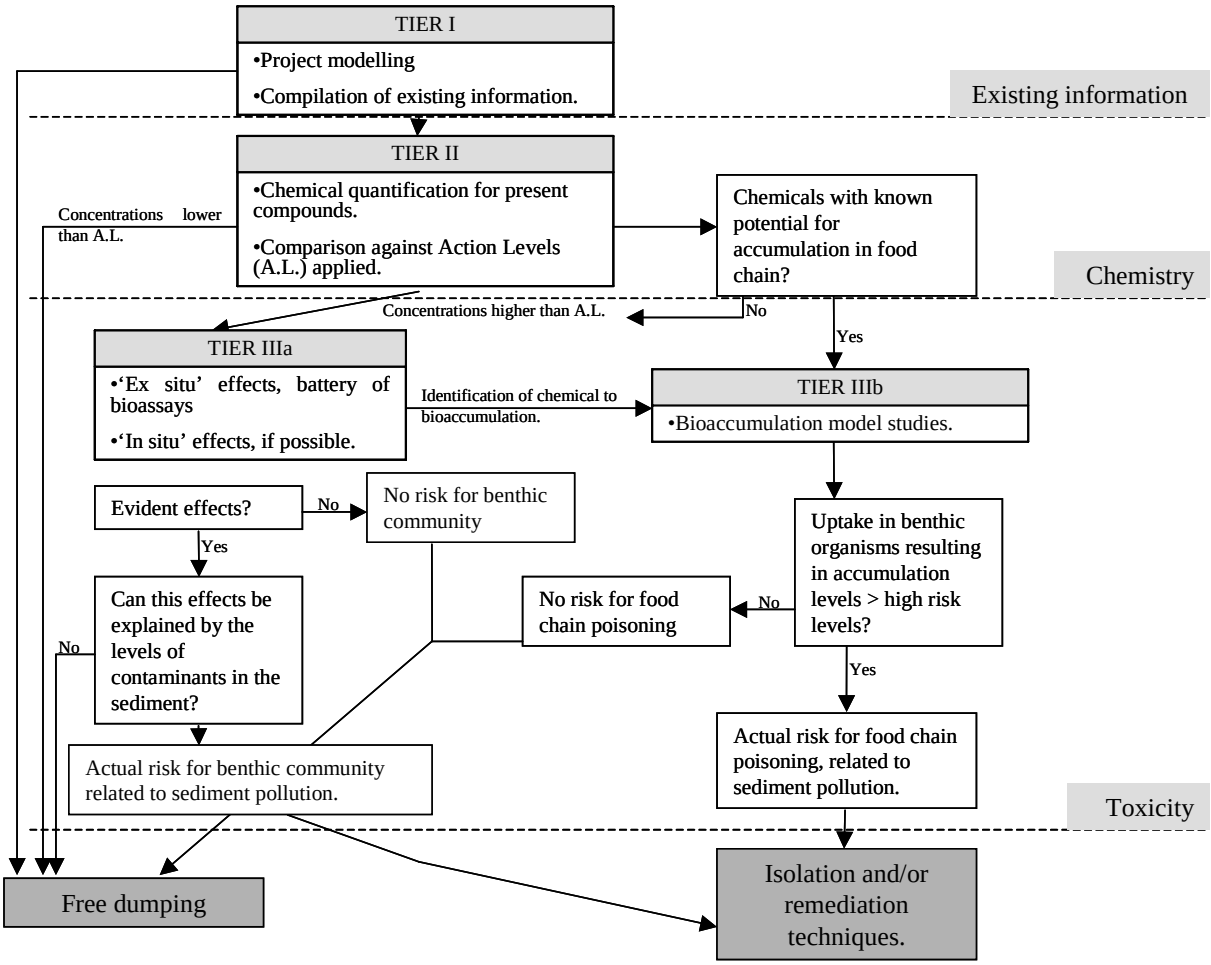

Figure 1. Dredged material management framework using physico-chemical and ecotoxicological endpoints (from Den Besten et al., 2003).

A second type of guidelines used for dredged material characterisation are the chemical benchmarks, namely Action Levels in the Spanish case (CEDEX, 1994). Despite there is some controversy in the last years about the development of chemical sediment quality guidelines (SQGs) and its use to support contaminated sediment and dredged material management (i.e. Jones Lee and Lee, 2005, Crane, 2003, Babut et al., 2003, McCauley et al., 2000, Chapman and Mann, 1999), they are still recommended for screening purposes on early tiers (PIANC, 2006). Spain uses two sets of SQGs to establish three different management categories: a first one for which "adverse effects are not foreseen in accordance with chemical concentrations below the

lower Action Levels; a second one for which open water disposal is not allowed according to the high chemical concentrations –above the higher Action Levels-; and a third cetegory that comprises intermediate chemical concentrations, for which further analyses are needed to proceed with the least threatening management option" (CEDEX, 1994). Thus, even if they are not effect-based chemical benchmarks, the ALs intend to predict sediment toxicity when chemical concentrations are below AL1s or above the AL2s.

According to the future actions included in the CEDEX report, these recommendations should be reviewed and adapted to the international standards (CEDEX, 1994). It is expected that the list of contaminants assessed is being adapted and upgraded with new arising anthropogenic compounds, such as PAHs or TBTs. In addition, biological endpoints are thought to be included on a secondary tier to evaluate the ecotoxicological characteristics of the dredged materials. Fig. 1 summarises an assessment framework in agreement with the former RRGMMD, but including the upgrades recommended by the scientific and regulatory communities described above. This framework follows through different tiers that are thought to inform the decision of whether open water diposal is a suitable managment option or not, but relies more on the separation of two different types of contaminants according to its potential for bioaccumulation and biomagnification through the food web. If the dredged materials being assessed present contaminants that may bioaccumulate and biomagnify, the process should proceed through a third tier, Tier IIIb, to address these particular processes by means of theorethical models and, if necessary, laboratory simulations to determine tissue concentrations in benthic organisms upon sediment exposure.

In the case of any contaminant included in the first group of compounds exceeds the corresponding AL1, a third tier (Tier IIIa), consisting on different biological endpoints, is used to assess for adverse effects on potential receptors. These may include infaunal organisms that are in direct contact with the sediment and/or ingest it for feeding purposes, but also organisms living in the water column that can be affected by the depletion of oxygen concentrations, suspended particles or through exposure to the contaminants that can be solubilised during the excavation and disposal works. This last type of test is especially recommended in countries such as Spain where water quality standards are not included in the general framework for dredged material management.

INVENTORY AND EVALUATION OF BIOLOGICAL TESTS FOR REGULATORY USE IN SPAIN

Despite several countries have already developed and standardised several biological toxicity tests for dredged material regulatory purposes, in Spain bioassays had been used only for sediment quality assessment but their use had not been standardised. Because judging when a biological test is ready for the regulatory evaluation of dredged material is based principally on the quality and quantity of the scientific information available (PIANC, 2006), a sound review of all previous experiences on biological testing around the world to develop an inventory of available methodologies (Table 1). The tests were organised in four different groups according to its possible application in the regulatory milieu: 1) tests that are suitable for an early screening tier; 2) tests performed on water only exposure, including pore water, sediment overlying water or sediment elutriates (liquid phase bioassays); 3) tests that consider the exposure to sediment-bound contaminants (solid-phase bioassays) and 4) tests designed to assess bioaccumulation potential from sediments. For each bioassay, the most recommended test species in Spain should be identified, taking into consideration that the test species already established in the Spanish laboratories are representative of the area being investigated and, a priori, they may be used for testing with dredged materials.

After this inventary was completed, the tests were evaluated for different attributes of the test itself and the test organism. Standardisation, commercial availability and cost-effectiveness of the test, compatibility and specificity of the measurable endpoints for a wide range of contaminants and interpretation, reliability and reproducibility of the results are some of the attributes that were considered positively. Regarding the test species, tolerant to a wide range of natural physico-chemical parameters considered as potential confounding factors (pH, salinity, grain size, organic carbon, volatile sulphides, etc.), and widely distributed and a easily handled and cultured organisms are preferred. Although it would be recommended to use organisms that are representative of those living in the particular disposal site, the use of specific organisms would require a prolonged research program to evaluate their use, causing unreasonable delays and expenses (Table 2). Despite scientists and stakeholders with strong expertise in sediment management were involved in this process, the rank of toxicity bioassays was principally subjective and adapted to particular circumstances. Detailed information on the evaluation of each test is available in DelValls et al. (2001).

Table 1. Inventory of bioassays used for sediment quality assessment and/or dredged material characterisation.

Bioassay	Species	Endpoint	Exposure route (type)	Exposure period	Guidelines	References
Microtox® (bacteria)	*Vibrio fisheri*	Inhibition of bioluminescence (IC_{50})	Screening (all)	5-30m	ASTM, EPS, RIKZ, NOAA, APHA, ISO, DIN	RIKZ, 2000; EC, 2002; ISO, 1997; APHA, 1995; ASTM, 1995.
Rotoxkit® (rotifers)	*Brachionus plicatilis*	Survival(LC_{50})	Screening	24 h	RIKZ	RIKZ, 1999a.
Pseudomonas sp.	*Pseudomonas fluorescense.*	Enzimatic activity	"	48 h		
Copepods	*Oncaea sp., Euterpina sp., Acartia clausii* (for stuarine samples)	Survival (LC50)	Liquid phase	48h	RIKZ, CEFAS, ISO	ISO, 1999; CEFAS, 1997; RIKZ, 1994a 1994b;
Fitoplankton	*Tetraselmis sp, Nannochloropsis gaditana, Chaetoceros sp., Chlorella sp.*	Growth	"	2-5d	OECD, ISO, APHA-AWWA-WPCF	OECD, 1998a; ISO, 1995; APHA-AWWA-WPCF, 1992.
Fertilization and larval development	*Paracentrotus lividus*	Fertilization (%), larval development	"	48h/ 96h	EPA, CETESB, OECD, RIKZ	RIKZ, 1999b; CETESB, 1999; US EPA, 1996, 1995; ASTM, 1986.
Rotifer population	*Brachionus plicatilis*	Population decrease (LT50)	"	7d		

Table 1. Inventory of bioassays used for sediment quality assessment and/or dredged material characterisation (Cont.).

Bioassay	Species	Endpoint	Exposure route (Type)	Exposure period	Guidelines	References
Amphipods	*Ampelisca sp., Corophium sp., Microdeutopus gryllotalpa*	Survival	Solid phase	10d	EPA, ASTM, CEFAS, RIKZ, ICES, EPS, PARCOM	EC, 2000; RIZK, 1999d; PARCOM, 1995; US EPA, 1994; ASTM, 1991.
Echinoderms	*Echinocardium cordatum*	Survival, burrowing activity	"	14d	RIKZ	RIKZ, 1999c.
Polichaetes	*Arenicola marina, Nereis sp., Neanthes sp.*	Survival, growth	"	10d/ 14d	PARCOM, ASTM, CEFAS	PARCOM, 1995; ASTM, 1994; Thain & Bifield, 1993.
Mysid	*Neomysis integer, Siriella armata*	Survival	"	10d	EPA, ASTM	US EPA, 1993; 1987; ASTM, 1990.
Benthic microalgae	*Cylindroteca closterium*	Growth	"	72 h		
Bivalves	*Ruditapes philippinarum, Scrobicularia plana, Venerupis pullastra, V. rhomboideus, Tapes decussates.*	Survival, burrowing activity, biomarkers, hystopathology	"	7d	EPA	USEPA, 1995.
Benthic and pelagic fishes	*Solea senegalensis, Scophtalmus maximus, Sparus aurata, Fundulus heteroclitus*	Survival, burrowing activity, biomarkers, hystopathology	"	96h-60d	PARCOM, OECD, EPA, ICES	ICES, 2000; PARCOM, 1995; US EPA, 1995; OECD, 1992.

Table 1. Inventory of bioassays used for sediment quality assessment and/or dredged material characterisation (Cont.).

Bioassay	Species	Endpoint	Exposure route (Type)	Exposure period	Guidelines	References
Bivalves	*Ruditapes philippinarum, Scrobicularia plana*	Bioaccumulation	All	28d	EPA, OECD	OECD, 1998b, ASTM, 1997.
Polichaetes	*Arenicola marina, Neanthes sp., Nereis sp.*	Bioaccumulation	All	28d	EPA, ASTM	US EPA, 1993; ASTM, 1997.

Table 2. Attributes considered for the evaluation of sediment toxicity tests.

Reliability	Evaluates the sensitivity of the bioassay to determine the toxicity associated with the exposure phase that is being evaluated (0: low sensitivity; 10 very sensitive).
Reproducibility	Detemines the probability of obtaining similar results on following testing for the same sediment samples (0: low reproducibility; 10: high reproducibility)
Standardisation	Determines if protocols have been evaluated by international agencies or previous scientific publications (0: not considered by international agencies and no guidelines available; 10: there is already a "cookbook" for the test)
Interpretation of results	Evaluates the difficulty of interpreting the test results (0: very difficult; 10: very easily interpreted).
Authocton species	Determines if the test species is authocton of Spanish coasts, if it is frequent at commercial or natural hatcheries. Additionally if the specieshave been tested for sediment toxicity assessment in Spanish litoral ecosystems was evaluated positively.
Ecological importante	Determines its importante on the trophic chain considered (0: not convenient; 10: describes perfectly the exposition route). The dificulty to establish the taxonomy of the species was evaluated together in this attribute.
Laboratory culture	Establishes the viability of laboratory culture of the test species (0: the life cycle cannot be completed under laboratory culture; 10: the stock of test organisms can be obtained from laboratory cultures).
Tolerance	Detemines the tolerante of the test organisms to variations in natural conditions such as salinity or temperature. The tolerance of the organism to potential confounding factors such as gran size have been also evaluated
Commercial	It determines the performance of the test by a private company or if the test is commercially available
Costs	Evaluates the relative cost of the tests have been evaluated.
Compatibility	Evaluated the compatibility of the endpoints with the exposure media. For example, it is more convenient to test a population of test organisms than a determined number of organisms or to consider two endpoints instead of one on the same test. Generally the compatility is higher for biomarkers of effect such as hystopathology followed by survival and other sublethal endpoints such as growth, reproduction or other biomarkers of effect. Moreover bioassays for which two endpoints are measured are preferred.

Later on, the paradigm for developing sediment toxicity bioassays for the regulatory assessment of dredged material published by Dillon (1994) was used to investigate for the gaps of knowledge that further investigations should address (Table 3). The commercial test Microtox®,that measures a sublethal endpoint in a population of the bacteria *Vibrio fischeri*, seemed a promising test as it was widely recommended for dredged material characterisation despite some differences in the exposure phases and the extraction procedures (Den Besten et al., 2003). Among the screening methods available, this test was considered the most sensitive, standardised, reliable and reproducible, and its commercial availability represented an important advantage over other tests such as the one using *Pseudomonas sp.*

Similarly there were some bioassays already recommended to test the water phases of dredged materials. The test using early life stages of different invertebrates was recommended for dredged material characterisation and was already established in several Spanish laboratories. The bioassay using rotifers, which evaluates the chronic effects on a whole population of a commercial organism, had been used in Spain for sediment quality assessment but was not supperted by its standardisation. Similarly, several mysid species had been widely used for water quality assessment but they had not been used for dredged sediments. Other bioassays, such as the test using copepods or phytoplankton, had an important disadvantage on the low reliability of results for sediment quality assessment, nonetheless both bioassays were standardised for water quality assessment (ISO, 1999; 1995).

Among all the tests used for whole sediment toxicity assessment, the bioassay using amphipods was by far the more referenced one. This bioassay, which is recommended worldwide for sediment toxicity assessment and especifically for dredged material, was performed in Spain using several species but their sensitivity had not been tested. The bioassays using different species of bivalves, fishes and polychaetes had been considered for sediment quality assessment (SETAC, 1993; OSPAR, 1995) and were already established in Spain (Riba et al., 2003; Saiz-Salinas & Francés-Zubillaga, 1997). On the contrary there was no information on testing with dredged materials and some controversy on test species sensitivity as these benthic species appear less sensitive than other benthic invertebrates such as amphipods (Batt and Raffaelli, 1998). On the other side, the bioassay using irregular sea urchins was though to be reliable, reproducible and was already standardised for its use on dredged materials (RIKZ, 1999; Stronkhorst, 2003). This bioassay, which was not established in Spain, had the main disadvantage on the difficulty to find the number of organisms needed to test a large set of samples, which in turn becomes more costs for testing. The lowest position corresponded to the bioassay using mysid species due to the low

number of references on dredged materials and the lower sensitivity of these species than other benthic organisms such as amphipods.

Considering that a set of two to four bioassays is recommended, including the measurement of lethal and sublethal endpoints in potential receptors of concern corresponding to different ecological and taxonomic levels. It seems that sediment toxicity assessment have relied untill now on survival, and this is specially true for dredged material assessment because managers and in general the regulatory community are still more confident on lethal endpoints (PIANC, 2006). Other sublethal endpoints such as growth and reproduction, and some sets of biomarkers are the subject of research at this moment (Martin-Diaz et al., 2004), but they are still on an early developmental stage. In relation to bioaccumulation tests, although the different agencies that include these tests for dredged material characterisation agree on measuring tissue residues of the contaminants of concern in bivalves and/or polichaetes upon laboratory exposure to the sediments being assessed (US EPA, 1998), these methodologies have had a limited application due to the high difficulty when interpreting the test results (PIANC, 2006).

LABORATORY RESEARCH

To elaborate on the different aspects of the test performance, namely to discriminate between harbours sediments that give rise to adverse effects and those that do not, a first research phase consisting on testing a wide range of dredged materials was carried out. This study considered three to four sampling stations at several commercial ports located along the Spanish coast: the port of Barcelona and Cartagena in the Mediterranean, the port of Cádiz and Huelva, in the South Atlantic coast, and the ports of Coruña, Bilbao and Pasajes in the Cantabric. The selection of these ports was based on regulatory and scientific judgements, namely the routine dredging activities performed by each port to maintain navigation or the known contamination problems in some particular areas (DelValls et al., 2003a). At each sampling station, bottom sediments would be characterised following the former recommendations for dredged material characterisation and, in addition, several biological tests would be used for their ecotoxicological characterisation. The questions being addressed for each bioassay would include:

1) the assessment criteria for the test performance,

2) the identification of potential modifying factors,

3) and the threshold limits used to classify the biological response.

Table 3. Developmental phases to be followed when developing new sediment bioassays for the regulatory characterisation of dredged material (extracted from Dillon, 1994).

Phase I: Initial development by test proponent	Phase II. Evaluation by multiple laboratories	Phase III. Development of a standard test method	Phase IV. Evaluation by user groups
i. Scoping	Continued research and development	Intertest comparisons	Joint agency consideration
Rationale	Interlaboratories studies	Verification/validation	Training with institutional "cookbook"
Assessment/measurement end points	Interpretative guidance	Peer-reviewed publications	Joint agency recommendations
Test end points	Testing with a wider range of dredged material	Protocol published by standard-setting group	Periodic review
Test species	Species sensitivity to major contaminants		
Literature review	Cost and logistics		
Alpha protocol	Peer-reviewed publications		
ii. Laboratory research development	Acceptance by the scientific community		
Statistical design	Gamma protocol		
Experimental design and procedure			
Quality assurance/ quality control			
Test ruggedness			
Dredged material testing			
Peer-reviewed publications			

Furthermore, testing in paralel would allow to make intertest comparisons not only on the relative test sensitivity and interpretative guidance, but also on the cost and logistics, and in general in all aspects related to the test itself and the biological responses. In this first laboratory research phase the toxicological responses under consideration included:

1) The Microtox® SP test, as an screening methodology for whole sediment toxicity.

2) The bioassay using polychaetes of the species *Arenicola marina*, the bioassay using *Echinocardium cordatum*, the bioassay using juveniles of the commercial clam *Ruditapes philippinarum* and the bioassay using several amphipod species to test for direct effects on benthic invertebrates. For this last test, the relative sensitivity of the regional species *Ampelisca brevicornis* and the widely recommended species *Corophium volutator* was investigated.

3) To assess for direct column effects, the toxicological responses on sea urchin embryos of the species *Paracentrotus lividus* and on a population of the rotifer *Brachionus plicatilis* were selected to test the elutriate phases of the sediments. This phase was selected instead of interstitial water or other organic extration because it is considered representative of dredging resuspension processes in the water column (Edwards et al., 1995; Alden III et al., 1982; 1987). In addition, interstitial waters are part of the sediment matrix thus are considered during the exposure to the whole sediment.

Finally, bioaccumulation of organic and inorganic compounds were studied by measuring tissue concentrations in the lugworm *Arenicola marina* and the clam *Ruditapes philippinarum* after exposure to the sediments in the laboratory.

To conclude with the laboratory research, different tests were the subject of evaluation through several inter-laboratory studies. This exercise considered the Microtox® SP test, the sea urchin embryo bioassay, the amphipod survival test and the test using juvenile clams. These four test were selected according to their developmental stage and their establishment in the Spanish laboratories.

RELATIVE PERFORMANCE AND FIRST RESULTS

After completing this research, a set of biological methods and guidelines was recommended (Table 4). The Microtox® SP test was considered a suitable screening method for an early tier as the standardisation of this commercial device allows to perform a large number of samples in few time (1 h each sample) and the all year round. Moreover,

this toxicological response was the most sensitive of all the bioassays tested when the Canadian guideline of 1000 mg L^{-1} is used as ecotoxicological criteria. Concerning this point, it seems recommended to collect a wide database to develop a suitable ecotoxicological guideline representative of the national standards, and to develop some correction for the proportion of fine sediments as the one developed by Stronkhorst (2003). Other protocols are being designed to reduce the effect of turbidity and colour that may be used if necessary (Campisi et al., 2005). When the Canadian guideline is used, some false positives of toxicity may appear related principally to the particle size of the sediments, but this test is still recommended on an early tier as these false positives of toxicity would be identified later on (Casado-Martínez et al., 2006a).

The bioassay using amphipods seemed the most recommended for regulatory uses among the tests using several benthic species. The two species considered, *Ampelisca brevicornis* and *Corophium volutator*, presented a similar incidence of toxicity when the statistical difference from a control sediment is used (about 70%) and a 10 % of misfit between contamination categories and toxicity. Despite some differences for low and medium-low contaminated sediments, which may be related to the shipping and handling of the individuals of *Corophium volutator,* and some adaptation of test organisms to non-chemical variables in some areas. Although it is difficult to elaborate on the relative performance of each test species for Category II materials, the use of regional species that may have developed some adaptation to non-chemical variables, may suppose an added value as being representative of the potential disposal site. Other non-chemical variables such as grain size and organic matter content of the sediments were studied in relation to the quality criteria already established for several amphipod species and the results indicate that both species have similar responses. In addition, these two amphipod species, *Corophium multisetosum* and *Microdeutopus gryllotalpa* have been cross-checked with acceptable results on interspecies and inter laboratory variability (Casado-Martínez et al., 2006b). According to these results, an ecotoxicological criteria established by the significant diference from a control or reference sediment is the most suitable indicator as threshold for toxicity. This criteria has the added advantage of differenciate between categories of toxicity by changing the statistical value "p".

The other whole sediment toxicity tests, namely the bioassay using the polychaete *Arenicola marina*, the clam *Ruditapes philippinarum* and the irregular sea urchin *Echinocardium cordatum,* were also performed succesfully in the laboratory for testing a wide range of dredged materials, although these species resulted in less sensitive endpoints than

amphipod mortality. Although these tests are not recommended for routine testing in Spain as these test species offer a little discrimination power between Category II materials, they are still useful for sediment quality assessments to address different exposure routes (filter/deposit feeders).

Among the two tests considered to assess for direct effects on the water column, the test using sea urchin embryos was more sensitive upon exposure to the sediment elutriates than the bioassay using rotifers (Casado-Martínez et al., accepted). This last test was affected by some positive effects related to high nutrient release during sediment elutriation while sea urchin embryos are sensitive to natural toxicants such as hydrogen sulfides or ammonia. The influence of such factors may be easily detected and cancelled out through proper TIE studies.

Table 4. Biological tests and guidelines for the ecotoxicological characterisation of dredged materials.

Bioassay	Endpoint	Toxicity criteria
Microtox® SP and BSP test	Decrease in luminescence	IC_{50} <1000 mg L^{-1} dry weight
Test using sea urchin embryos of species *Parecentrotus lividus*	Abnormal larval development	Mean percentage of abnormal development>25% and statistically different from controls.
Test using amphipods (different species).	Mortality	Mean mortality >25% and statistically different from a control or reference sediment.
Bioaccumulation studies (different species).	Concentrations of key compounds	Concentrations measured in the tissues of key benthic organisms is higher than the concentrations measured in organisms under reference conditions.

Although the results of this test in the interlaboratory exercise were satisfactory, they still evidenced the importance of standard protocols to obtain this aqueous extraction and the need of common toxicity criteria for the good performance of this toxicological response due to its subjectivity (Casado-Martínez et al., 2006c). As for the other bioassays, an ecotoxicological criteria established by the significant diference from a control population is the most suitable indicator as threshold for toxicity. This criteria has the added advantage of differenciate between categories of toxicity by changing the statistical value "p".

Concerning the bioaccumulation tests performed on dredged sediments, individuals of the polychaete *Arenicola marina* and the bivalve *Ruditapes philippinarum* have been measured for whole body concentrations of several metals including As, Cd, Cr, Cu, Hg, Ni, Pb and Zn, PCBs and PAHs. The results showed a clear increased bioaccumulation of metals and PCBs in the organisms exposed to the highest sediment concentrations. The metal concentrations in sediments were able to predict bioaccumulation, although it is difficult to establish an straighforward relationship between the concentrations in sediments and in lugworms in the intermediate range of concentrations. Among other factors, the organic matter content of the sediment seems to be determining in the bioavailability of contaminants bound to dredged muds, and so it is for PCBs. On the contrary, the results of PAHs depicted a more complex process of bioaccumulation of these compounds in the polychaete *A.marina*. For the organic compounds, the calculated BSAFs were compared to the standard value of 4 recommended for screening purposes and our results were most often below these value. Although the number of cases studied for organic compounds is considerably lower than for other metals, the highest concentrations presented for the compounds that are more environmentally threatening indicate the need for assessing the bioaccumulation potential from particular compounds to benthic invertebrates in relation to possible biomagnification processes.

RECOMMENDATIONS FOR USE

As it was previously discussed, biological tests are recommended as part of the characterisation process for dredged material management in use although at this moment, they are not use for routine testing. These methodologies were especially recommended for Category II materials, as they cannot be classified definitely through the physico-chemical analyses alone. Generally, a set of two to four bioassays is recommended internationally for dredged material characterisation, including several potential receptors of concern representative of different ecological and taxonomic levels (PIANC, 2006). Although it is not understood as the best tool for sediment toxicity assessment, a test set comprising the Microtox® SP test, the sea urchin embryo bioassay and the amphipod mortality test represents a feasible approach that accomplishes the minimum requirements for the regulatory characterisation of dredged materials, including exposure routes that are direct and relevant of potential toxic effects according to the assessment questions foreseen in the RRGMMD. The data base available on the relative performance of this test set is scarce, although an early data base on physico-chemical and biological properties of harbour

sediments from Spanish ports is available after different laboratory investigations. These data indicates that dredged sediments are principally organic matter-enriched muds, with the exception of few coarse sediments. These coarse sediments presented always low contaminant concentrations, which supports the application of the grain size criterium on early tiers to avoid the chemical analyses. On the contrary, the muddy sediments were affected by metallic and organic compounds varying on a whole range of concentrations. The presence of important anthropogenic sources of contamination, such as mining and industrial activities, was always related to high sediment contamination, nonetheless the harbour itself represents a secondary source of contamination that gains relevance in the absence of anthopogenic pressures in the surrounding areas (Casado-Martínez et al., 2006d).

Regulators and contracting parties are usually averse to the introduction of biological tests in decision-making as are thought a new barrier for the economic development of the harbour that may increase considerably the costs of pre-dredging investigation and the economical resources allocated for dredging activities. Nonetheless these judgements are senseless until source control measures are not effective in reducing the chemical load of harbour muds because, in most of our samples, the chemical measurements alone would be enough to judge that open water disposal is not a suitable management option. In ports where the harbour itself represents the main contamination source, it is more probable that dredged materials fall into Category II, for which biological tests may gain relevance in decision-making. In this case, the use of an additional line of information may suppose important environmental benefits if the tests performed address properly the assessment questions. For doing this, both the whole sediments and the elutriates must be tested as a measure of direct effects the water column and on benthic organisms.

The results of the Microtox® SP test were similar to those obtained from the physico-chemical measurements, and despite the grain size of sediments appears as a major factor determining this toxicity endpoint the results correlate well with the mean contamination of the sediments. While amphipod mortality seems related to the presence of organic but principally metallic sediment contamination, the elutriate embryotixicy is not directly related to the concentrations in sediments as the toxicity registered for undilutes elutriates is largely dependent on the different solubility of sediment-bound contaminants. This results supported the need of some bioassay for testing aqueous extractions when water quality standards are not developed along the assessment framework, since SQGs are not a good predictor of the mixing processes occurring between waters and sediments.

The ecotoxicological characterisation of dredged sediments also pointed out the absence of tests that address properly the effects of PCBs and PAHs. This has been previously stressed by Stronkhorst (2003) for PCBs but also for dioxins and dioxin-like compounds, which is the reason of specific biological endpoints to assess for toxic effects related to these persistent compounds (i.e. DR-CALUX, Ah-Immunoassay). In Spain these type of techniques are not still established, although it seems recommendable to work on these new tests for these unaddressed toxicants (PIANC, 2006). Untill they are not fully standardised and validated, these compounds should be addressed through the chemical measurements in sediments and in biota to investigate for potential bioaccumulation and biomagnification through the food web. Although it is still difficult to interpret the results of chemical concentrations in tissues, especially concerning its ecological significance, five questions resume concisely what needs to be addressed for decision-making (Peddicord et al., 1997):

1) What is the toxicological importance of the contaminants whose bioaccumulation from the dredged material exceeds that from the reference material?
2) By what magnitude does the bioaccumulation from the dredged material exceed bioaccumulation from reference material?
3) What is the propensity for the contaminant to biomagnify within aquatic food web?
4) What is the magnitude by which contaminants whose bioaccumulation from the dredged material exceeds that from the reference material also exceeds the concentrations found in comparable species living in the vicinity of the proposed disposal site? And
5) For how many contaminants is bioaccumulation from the dredged material greater than bioaccumulation from the reference material?

It is worthy mentioning that our results evidenced some misfits between sediment contamination and toxicity for sediments falling into Category III. The difficulty of interpreting these results has been previously discussed (O'Connor et al. 2000) although Chapman and Anderson (2005) have already included some rules that apply in the case of contradictory results between the chemical and the ecotoxicological characterisations. The following rules apply: 1) sediment chemistry data will not be used alone except in those cases where extremely elevated concentrations where adverse biological effects are likely and 2) decisions will be based primary on biology not chemistry. It means that dredged

materials that are not toxic to multiple tests are not needed of the chemical analyses because there is no evidence of direct toxic effects.

CONCLUSIONS

A battery of bioassays has been identified and validated for the characterisation and management of dredged materials from Spanish ports. Nonetheless, assessment tools undergo a maturation process as knowledge and experience is gained regarding their performance and reliability under the variable conditions involved in their application (PIANC, 2006). Thus it is expected that the confidence of these techniques in decision-making will be increased through routine testing on a wider range of dredged materials. In addition, the development of sediment bioassays is not an static process and new developments are in progress. A good exchange of information between the scientific and regulatory bodies is fundamental to incorporate new improvements and for a good feed-back in the general paradigm for the development of biological testing.

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LINKING PHYSICOCHEMICAL AND ECOTOXICOLOGYCAL ASSESSMENTS FOR DREDGED MATERIAL CHARACTERIZATION IN PORTS AFFECTED BY METALLIC POLLUTION[1]

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Abstract

Surface sediments from two ports affected by mining activities (Cartagena and Huelva) were characterised following the traditional physicochemical characterisation based on contaminant concentrations together with laboratory toxicity tests. The toxicity tests included acute and chronic methodologies both on the whole sediment and on the sediment elutriates. As expected sediments reported remarkable concentrations of metals, some failing the higher limit values for open water disposal, and organic contamination in some areas affected by industrial and shipping activities. The toxicity assessment results showed differences among the two studied zones: the port of Huelva reported significant toxicities both for the whole sediment and the elutriates tests but the sediments from the port of Cartagena reported significant toxicity only for some whole sediment bioassays. These sediments provoked little or no adverse effects to other benthic species and similar responses to controls for elutriate tests. These results show that SQGs are not always a good predictor for sediment toxicity, especially to evaluate the risks of elutriate waters. In this sense the advantages and disadvantages of laboratory toxicity tests for dredged material characterisation and its use in ecological risk assessment for decision-making is further discussed.

KEYWORDS: SEDIMENT QUALITY ASSESSMENT, BIOLOGICAL EFFECT BASED ANALISIS (BEBA), BIOASSAYS, CONTAMINATED SEDIMENTS.

[1] Terra et Aqua (En prensa)

INTRODUCTION

During the last decades a number of international conventions on marine environmental protection have encourage impact assessment to evaluate potential effects on human health, living resources, amenities and other legitimate uses of the sea due to dredged material disposal (Neville-Burt and Hayes. 2005). Even though the greater proportion of dredged materials is similar in environmental terms to the sediments that are present naturally, a small proportion of sediments is contaminated and may represent a real threat. The extent of sediment contamination is largely influenced by operations carried out in ports and waterways such as passenger traffic, goods shipping, "accidental" spills or "intentional" discharges occurring close to navigational routes and despite point source control measures have significantly contributed to reduce sediment contamination in the late years, short and long-term sources, as the result of past and present activities, may have contributed critically to worsen the environmental quality of littoral ecosystems. The anthropogenic substances accumulating in aquatic systems can be distinguished into two groups: nutrients and pollutants (Goossens and Zwolsman, 1996). Among others metals, metalloids, oil and grease, hydrocarbons and pesticides are pollutants traditionally found in ports and waterways, though the nature of the activities performed, the characteristics of the area and the control measures adopted determine the environmental quality of the aquatic system.

A great proportion of dredged materials needs to be disposed of into the same aquatic system due to economical, technical or logistical reasons. This management option is considered if sea disposal is identified as the least detrimental option according to the characterisation of the sediments to be dredged and after completing the dredged material management framework. One of the most simple dredged material assessment frameworks was given by Neville-Burt and Hayes (2005) and the subject of pre-dredging investigations for materials characterisation to evaluate the environmental aspects of dredging operations have been addressed in several guides and recommendations set up by different groups of experts, such as the one from the IADC-CEDA series on environmental aspects of dredging (Peddicord and Dillon, 1997) or the one recently published by PIANC (2006). These guides are thought to lead the reader through a "highly focussed, cost-effective evaluation of the potential environmental impacts of dredging operations" that can be summarised in four steps (Peddicord and Dillon, 1997):

- **Step 1:** Project planning, including the nature and scope of the activities, the potential dredged material placement options and the regulatory requirements.
- **Step 2:** Initial evaluation, where available data is examined may lead to the conclusion that no further pre-dredging evaluations are needed is gathered if needed, one proceeds to Step 3.
- **Step 3:** Physical, chemical and biological characterisations of dredged material.
- **Step 4:** Interpretation of results of the data assembled and evaluated.

This paper looks at the methodologies and endpoint measurements involved during dredged material characterisation in relation to environmental risk assessment and dredged material management frameworks. Specifically the use of biological tests in the context of navigational dredging is addressed in two case studies consisting of sediments from two areas with known metallic contamination. An integrated approach, designed to meet the international recommendations on the application of biological tests for dredged material characterisation and management (PIANC, 2006), has been used to characterise harbour sediments and the results are presented to study the uncertainties on the use of these methodologies. The results cannot address the questions related to the project itself (Step 1 and 2) due to its hypothetical nature but can be studied to improve the use of effect based decision making.

STUDY AREAS

The ports included in this study are allocated in areas affected by important mining activities: the port of Cartagena and the port of Huelva (Fig. 1). The port of Cartagena is located close to the city with this same name in the South West coast of Spain. This city has been under the influence of an abandoned Pb-Zn mining district, which origins date back to the Roman empire age. This region became one of the most representative open cast mining areas in Spain after the middle 20th century that led to an intensive movement of metals that ultimate entered the aquatic environment through direct and indirect deposition. The ore vein was mainly composed of galena (PbS) and sphalerite (ZnS) with other minor elements such as Ni and Cd (Marguí et al., 2004). Furthermore this city experienced some industrial development during the 1960s and nowadays there are several chemical and metallurgical factories allocated in the surroundings of the harbour facilities - an electrolytic Zn plant, different fertilisers plants, a Pb smelter closed from March 1992, a fertilizer plant closed from 1993, a power plant, an oil refinery and a ship-yard.

The second area under study is the port of Huelva, located in the South Atlantic coast of Spain. The Ría of Huelva comprises the estuary of the rivers Tinto and Odiel, which form the Padre Santo Channel (Fig. 1). The area delimitated by these two rivers is characterized by important mining and metallurgical activities dated 3000 years old and based on pyrite (FeS_2) and other sulphuric minerals. This estuary suffered from continuous metal discharges over centuries throughout acid mine drainage and solid wastes, which represent an important long-term contamination source. Three industrial areas are present in the Tinto and Odiel catchment areas: a first one upstream the Tinto river, a second one located in the Odiel river before it joints the Tinto and a third one just after this confluence. These areas include a cellulose factory in the Tinto river catchment area, which produces high quantities of pyrite ashes, different phosphates and fertilizers plants, a copper and sulphuric acid factory and a power plant, a petrol refinery and different chemical plants are located in the left margin of the channel.

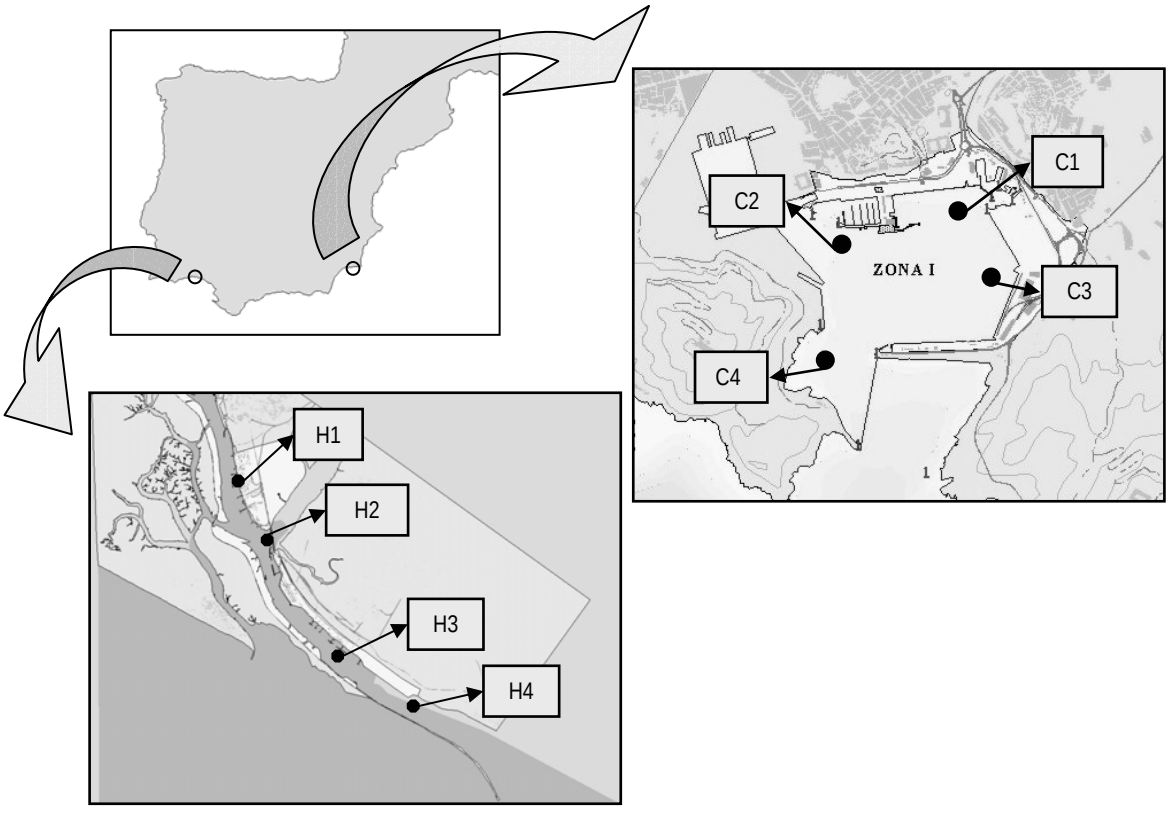

Figure 1. Sampling stations at the ports of Cartagena (C#) and Huelva (H#).

APPROACH

Sediment sampling

The sediment samples were collected in April 2003 with a 0.025 m^2 Van Veen grab from approximately the top 20 cm. On arrival to the laboratory sediments were homogenized and stored at 4ºC and darkness prior to analysis. For each study area, CEDEX and the University of Cádiz selected four sampling stations (Fig. 1). As Figure 1 shows, two inner stations were selected in Cartagena, a fist one on the east (C1) and a second one in the western bay (C2), and other two stations were located on the external part of the bay (C3 and C4). In Huelva four different stations were sampled and numbered seaward along the estuary.

Physico-chemical characterisation

The characterisation of sediments was performed on sediments dried at 40ºC for 24-h and followed the CEDEX Recommendations for Dredged Material Management (1994). Grain size distribution followed UNE 103 101 and total organic matter content was estimated by loss of ignition (LOI) at 550ºC and gravimetric determination, as recommended for small dredged volumes. Metals were determined in microwave acid-digested samples. The concentrations of Cd, Pb, Cu, Zn and Cr were determined using flame or furnace atomic absorption spectrometry, depending on the metal content. Mercury was determined using the cold vapour technique and for As the hydride generation technique was chosen before quantification using atomic absorption spectrometry.

PCB congeners #28, 52, 101, 118, 138, 153 and 180 and polycyclic aromatic hydrocarbons (PAHs) were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment,before concentration and clean-up with column chromatography. Determination of PCBs was made with gas chromatography with electron capture detection (GC-ECD) (EPA 8080) and 12 PAHs (acenaphtylene, acenaphtene, anthracene, benz(a)anthracene, benz(a)pyrene, chrysene, dibenz(a,h)anthracene, phenanthrene, fluoranthene, fluorene, naphthalene and pyrene) were determined with HPLC with fluorescence detection (EPA 8310). Detection limits were 0.8 and 10-30 μg kg^{-1} dry weight of sediment of PCBs and PAHs respectively.

Recoveries of analytes determined ranged from 60% to 120% and all the analytical procedures were checked with reference materials and allow agreement with certified values.

Ecotoxicological characterisation

Bioassays are among other things required for the characterisation of the toxic potential of dredged sediments and for environmental risk assessment of the disposal of dredged material. For that reason marine bioassays are also recommended in several dredged material management guidelines (Peters et al., 2002). These guidelines recommend sensitive and standardized sediment-dwelling or sediment-associated test organisms that are reasonably similar to those found -or expected to be found- at the site (Chapman and Anderson, 2005) to assess acute and chronic toxicity. Generally a set of 2-4 bioassays with different taxa are recommended to assess acute toxicity. If biological tests are used to clarify gaps of information in decision-making the selection of test species and endpoints should include sensitive organisms (ecological receptors) in the environment that may be exposed to the contaminants and should address all the exposure pathways that may operate to bring contaminants into contact with the receptors. In the particular Spanish case, the framework assumes that the general goal of the assessment is to determine whether a dredged material, proposed for open-water disposal, is likely to cause adverse impacts at the disposal site. Thus the potential receptors of concern include invertebrates that live in the sediment, animals and plants living on the sediment surface, bottom associated fish, pelagic fish and invertebrates, birds and other wildlife, and humans using the site (PIANC, 2006).

The biological tests performed in this study are summarised in Table 1. Direct benthic effects were assessed in the amphipod *Corophium volutator*, the polychaete *Arenicola marina* and the irregular sea urchin *Echinocardium cordatum*. These three organisms are infaunal benthic species in direct contact with the sediment where they are buried. In addition the clam *Ruditapes philippinarum* was included to assess the potential effects of sediment resuspension events. This commercial clam, also known as the Manila clam, is an infaunal bivalve that lives buried in the sediments. On the contrary this species is a filter-feeder feeding on the overlying water thus it address specifically direct water column effects. To complete the assessment of direct water column effects the sediment elutriates were tested for toxicity in sea urchin embryos and rotifers. In this way the test set includes

both acute and chronic exposure (i.e. acute are the 10/14-d tests and chronic the 7-d rotifer population decay test; the embryogenesis success is considered a sub-chronic endpoint) and lethal and sublethal endpoints (i.e. survival and burrowing activity). In addition bioaccumulation potential of compounds that are known to bioaccumulate and biomagnify in aquatic food webs, such as PCBs or mercury, was evaluated by measuring the residue concentrations in clams after the standard 28-d exposure and lugworms after the 10-d of exposure. Finally the results of the Microtox® device following the standard protocol for soil and sediments SPT were considered due to its potential suitability to screen for toxicity in dredged sediment samples.

Table 1. Bioassays performed for the ecotoxicological characterization of sediments.

Test species	Exposure route	Exposure time	Endpoint
Vibrio fischeri	Whole sediment	30-min	Bioluminescence inhibition
Corophium volutator	Whole sediment	10-d	Survival
Arenicola marina	Whole sediment	10-d	Survival
Echinocardium cordatum	Whole sediment	14-d	Burrowing/survival
Ruditapes philippinarum	Whole sediment	14-d	Burrowing/survival Bioaccumulation
Brachionus plicatilis	Elutriate	7-d	Population decay
Paracentrotus lividus embryos	Elutriate	48-h	Embryogenesis success

Data treatment and interpretation

The results of the physico-chemical measurements were studied in relation to the guidelines recommended in Spain for dredged material management. These guidelines follow an action level approach based on the use of two different limit values (the so called Action Levels –ALs-) that are used to classify the sediments in three different management categories. Despite the higher complexity of the classification process we have compared the chemical concentrations measured in the fine fraction (<63 μm) of the sediment samples with the national ALs for dredged material characterization (Table 2), in this way it was possible to identify the category for each sediment and the contaminants of concern in each area under study. The biological endpoints were studied in relation to the negative toxicity controls carried out with each batch of experiments. This control consisted of a sediment free of all contamination and toxicity for the solid phase bioassays and clean sea

water for the elutriate tests. A difference of 20% between the controls and the test and reference sediments is neither different nor environmentally relevant in short-term (e.g. 10-d) acute tests thus, if all sediment toxicity endpoints are ≤20% different from the reference, the sediments are not considered toxic even if the difference is statistically significant. As an screening test, the Microtox® results were compared with the Canadian limit value established at 1000 mg/L d.w. for dumping licensing (EC, 2002). Statistical analyses were performed by means of the statistical program STATISTICA®.

Table 2. Contaminants determined in sediments and Action Levels used for dredged material management (CEDEX, 1994). All values expressed in mg kg^{-1} except PCBs, expressed in μg kg^{-1}.

Compound	Action Level 1	Action Level 2
As	80	200
Cd	1.0	5.0
Cr	200	1000
Cu	100	400
Hg	0.6	3.0
Ni	100	400
Pb	120	600
Zn	500	3000
Σ_7-PCB	30	100

CASE STUDY 1: HUELVA

The sediments from Huelva reported significant differences in the proportion of fines, the organic matter content and the chemical load showing a clear decreasing trend along the estuary (Table 3). The inner station (H1) was characterised by the highest proportion of fines and organic matter and also the highest concentrations for most chemical compounds while H4, the station in the external estuary, was a typical coarse sediment free of all contamination. Stations H1 and H2, that are actually more influenced by the rivers, consisted of fine sediments rich in organic matter with As and Cu concentrations higher than the corresponding AL2 for aquatic disposal authorization and intermediate concentrations of Hg, Pb and Zn (Table 4). The higher concentration of Ni reported for station 3, located close to a petrol refinery, and evidenced the importance of addressing point sources in the general assessment framework. The organic micropollutants also identified some enrichment in the inner estuary and PCBs were the only organic compounds detectable in the two inner stations (H1 and H2).

In general the ecotoxicological characterisation of harbour sediments from Huelva was in agreement with the results of the physico-chemical analyses. The IC50 values obtained from the Microtox® device identified the two inner sediments (H1 and H2) as potentially toxic (Fig. 2). These sediments reported the highest contaminant concentrations in sediments although the plot of IC50 values indicate that some factor, for which the proportion of fines or the organic matter content accounts for, may be determining the performance of this endpoint. These results are in agreement with previous studies that reported the highest toxicities for the inner sediments and are explained by the high precipitation of metals in this area (Usero et al., 2001). Precipitation of metallic species such as sulphates or carbonates, easily bioavailable due to the weak links that bound these metals to sediments, occurs in the lower Odiel and Tinto rivers as a consequence of changes in pH and salinity. This precipitation is higher in the inner part of the estuary and decreases in intensity going seaward due to the gradient in the variables controlling these processes (Usero et al., 2000). Nonetheless the proportion of fines sediments has been identified as one of the main factors related to false positives o this endpoint -defined as the samples that are considered toxic by this test but do not cause toxicity to other test organisms-. This factor should be taken into account when interpreting the results for these two samples. On the contrary a high proportion of sand may cause false negatives -defined as the samples that are considered not toxic by this test but cause significant toxicities to other test organisms- (Ringwood et al., 1997; EC, 2002), which in turn could may have influence the results for H3. Despite false positives are less relevant from an environmental point of view since these samples can be properly addressed under a tiered approach, false negatives of toxicity may be considered an important drawback for a screening test.

High toxicities in the rest of tests supported the potential toxicity identified for the inner fine sediments during the first screening. The sediment with a higher proportion of sands, that was not a positive of toxicity for the Microtox®, did cause toxic effects and evidenced the importance of sediment properties when interpreting the results of the Microtox® assay. Considering the test species and the exposure routes addressed by each endpoint, in general the dredged materials from the Ría of Huelva may pose a risk through contact with the whole sediments but also through exposure to the sediment elutriates. Some decrease in elutriate toxicity for the inner sediments (H1) indicates that organic matter may decrease the bioavailability of contaminants by decreasing its solubility to the water phases. This factor may influence the results of elutriate toxicity tests when using organisms such as rotifers, that should be in starving conditions but may obtain extra food

from elutriates. On the contrary this factor is not relevant for echinoderm larvae because they do not need extra food (Apitz et al., 2005), which supports the lower solubility of contaminants from this inner sediment.

The results of the bioaccumulation tests showed that polychaetes bioaccumulate As, Cu and Ni to concentrations higher than those reported after exposure to a reference sediment, while clams bioaccumulate high concentrations of Cu, Zn and Pb under laboratory exposure to the dredged sediments (Fig. 3). Even if a significant elevation in the tissue concentrations does not necessary mean that risks to upper trophic levels are likely, it is reasonable to conclude from a failure to statistically distinguish the dredged material and reference exposed organisms that risks to upper trophic levels are unlikely (PIANC, 2006). Considering that the extent of bioaccumulation at higher trophic levels in the food chain is unknown, further assessments (e.g., trophic transfer modelling and dose calculations) should be also considered for those compounds with known biomagnification potential if there may be some transfer from sediments.

CASE STUDY 2: CARTAGENA

The sediments from Cartagena were more similar in grain size and organic matter content than the sediments in the previous case study, and they formed a clear gradient of sediment properties (Table 3). The chemical characterisation evidenced that dredged materials from Cartagena are affected by a "cocktail" of contamination consisting of several metallic and organic compounds at different concentrations. Dredged materials from Cartagena would fall into category III with high Cd, Cu, Pb, Hg and Zn concentrations. These sediments also reported high PCBs and detectable concentrations of PAHs. The Microtox® indicates potential toxic effects for all sediments except C3 (Fig. 2), that reported the lower proportion of fines, while the rest of tests reported very variable toxicity for the different endpoints measured. All sediments were toxic to amphipods and, at the highest sediment concentrations, toxicity to polychaetes also occurs (samples C1 and C3). On the contrary the sediments did not evidence neither elutriate toxicity nor lethal and sublethal effects on clams. Considering the results obtained in Huelva it seems that the contaminants bound to sediments from Cartagena are not bioavailable through exposure to the water phases, although the chemical composition of elutriates was not studied. Nevertheless, the bioaccumulation tests evidenced the increase in tissue concentrations of

Cd, Cu, Hg, Pb and Zn when the concentrations are compared to those measured in clams exposed to a reference sediment.

Table 3. Grain size and organic content of the sediments (g·kg^{-1}).

Sample	% coarse	% sand	% fines	Organic content
H1	0.07	9.71	90.22	20.27
H2	0.19	9.60	90.21	10.64
H3	0.03	56.02	43.95	6.30
H4	80.34	19.65	0.01	1.00
C1	3.95	38.24	57.81	10.54
C2	5.22	53.59	41.19	9.12
C3	0.93	67.20	31.87	7.19
C4	0.90	50.01	49.10	9.87

Table 4. Results of the physico-chemical characterisation. All values expressed in mg kg^{-1} except PCBs, expressed in μg kg^{-1}.

	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	PCBs[a]	PAHs[b]
H1	840	4.35	32.9	1938	2.38	34.6	383	2458	200	n.d.
H2	531	2.50	24.1	1497	1.99	7.10	385	1857	229	n.d.
H3	273	1.32	8.13	772	1.20	129	217	1176	n.d.	n.d.
H4	4.70	n.d.	9.70	1.90	0.04	0.80	5.30	20.9	n.d.	n.d.
C1	101	98.5	66.6	666	136	29.0	1397	8661	123	0.91
C2	64.7	17.5	45.6	313	32.7	15.3	748	1885	468	1.03
C3	88.0	31.9	57.6	453	115.2	19.3	1397	3310	108	0.66
C4	62.6	6.79	29.5	171	21.6	19.3	487	901	119	1.24

*n.d. means not detected or lower than the corresponding detection limit;
[a] Σ_7-PCBs;
[b] Σ_{12}-PAHs.

LINKING SEDIMENT CHEMICAL AND ECOTOXICOLOGICAL CHARACTERIZATION

Several approaches are used to link sediment chemical and ecotoxicological endpoints that can serve to incorporate biological endpoints in dredged material management. Three questions resume concisely how to address sediment toxicity assessments and the information arising: 1) are contaminants of concern present in the sediment and at which levels? 2) are these contaminants bioavailable? 3) are these contaminants causing adverse biological effects?

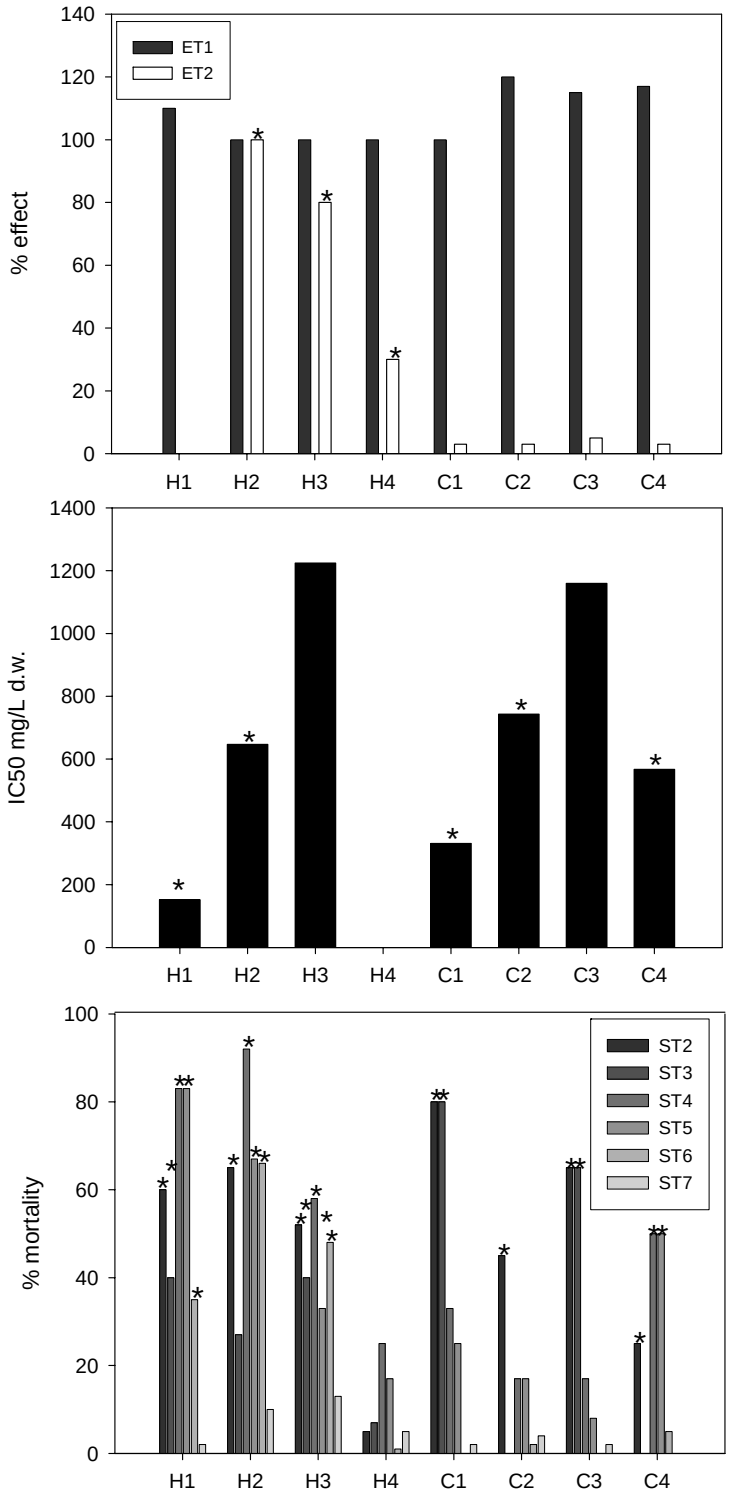

Fig. 2. Results of the ecotoxicological characterization of the sediments studied. Toxicity is identified with an asterisk. ET1: rotifer population decay expressed as % effect compared to controls; ET2: percentage of abnormal sea urchin larvae; ST1: Microtox® SPT as IC50 mg/L d.w. basis; ST2: % amphipod mortality; ST3: % polychaete mortality; ST4: % *E.cordatum* mortality*;* ST5: % not buried *E.cordatum;* ST6: % mortality *R.philippinarum*; ST7: % not buried *R.philippinarum.*

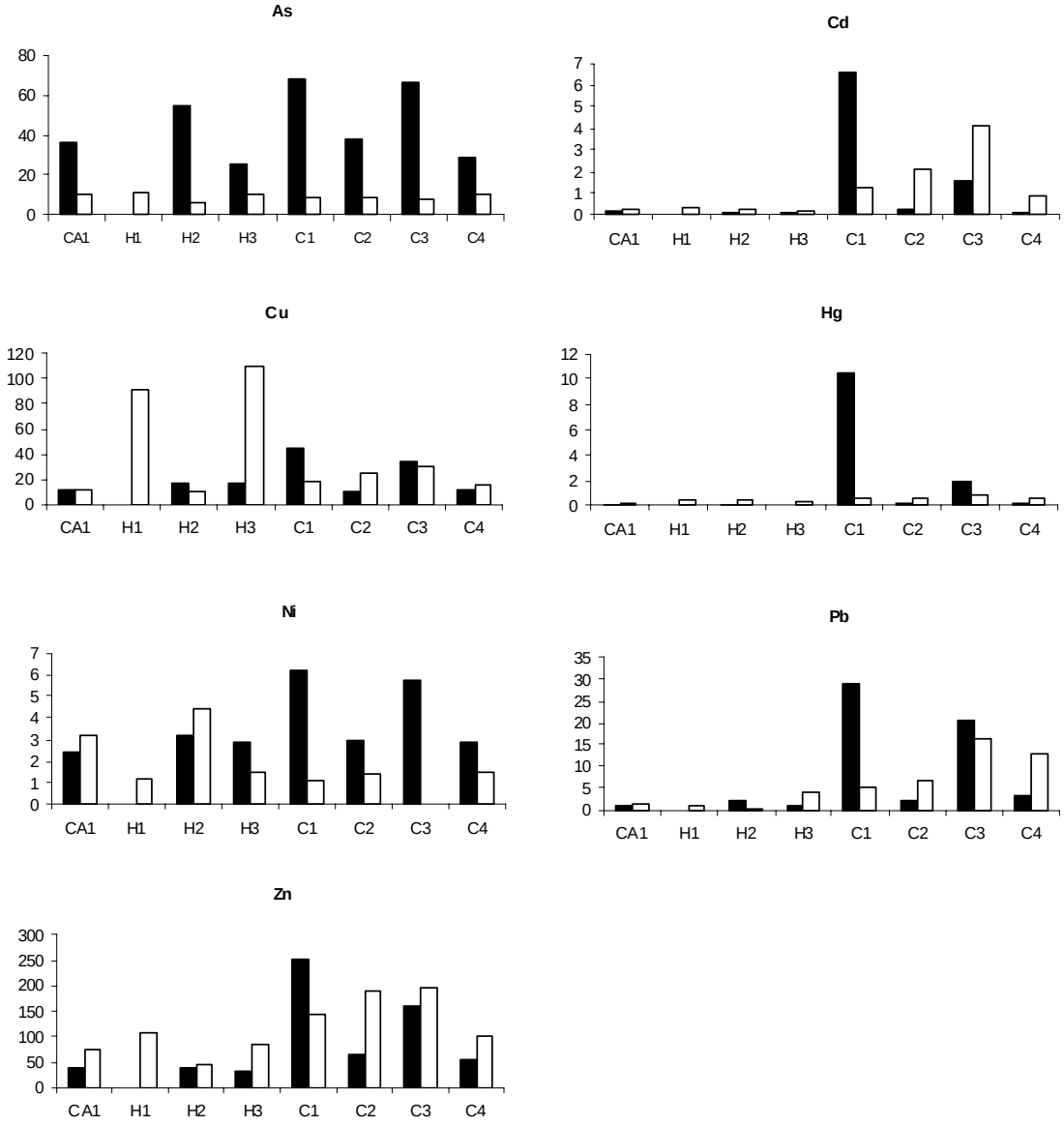

Fig. 3. Tissue concentrations of metals in polychaetes (*Arenicola marina*; black bars) after 10 days of exposure and clams (*Ruditapes philippinarum*; white bars) after 28 days of exposure to dredged sediments and a reference sediment (CA1). All values expressed in mg kg^{-1}d.w. basis.

The results are evident in the two ports studied (Table 5) and, even if it is not possible to identify the causes of toxicity, a significant reduction on survival of different benthic organisms occurs that evidences the high bioavailability of sediment-bound contaminants. If we consider these results in the general framework for dredged material management, this type of tests are not probably considered when a tiered-action level approach such as the one recommended in Spain is used. When the chemical concentrations are high, dredged sediments are not needed of further ecotoxicological assessments to decide if they are suitable for open water disposal or not, and the corresponding management strategy is selected according to sediment chemical concentrations alone (Table 6). In this study the ecotoxicological characterisation supports the results of the physico-chemical characterisation and the overall toxicity could be considered significant because multiple endpoints exhibit major toxicological effects, and these effects could be in some way related to sediment-bound contaminants. Nonetheless, and despite the physico-chemical approach seems useful to identify the contaminants of concern and even identify toxicity "hot spots" (Long et al., 2000; Casado-Martínez et al., 2006), certainly the physical, chemical and biological inter-relationships of sediment/water complexes are far too complex to be evaluated through a rather simplistic approach. This was especially true in the port of Cartagena possibly due to the higher complexity of this harbour, which determined there was no straightforward relationship between sediment contamination measured in the fine fraction and toxicity (Table 5). Our study and other studies support the use of dredging simulation by elutriate tests to predict concentrations of metal released to the water column from contaminated sediments, and furthermore to predict effluent and surface water quality during predisposal evaluation of dredged material intended for upland disposal (Edwards et al., 1995; Alden III et al., 1982; 1987). In addition, other benthic organisms whose sensitivity have been validated should be used to assess direct benthic effects in case the sediment chemical concentrations are not enough for decision making, but also to determine the general patterns in the bioavailability of sediment-bound contaminants.

Table 5. Results of dredged material characterization.

Sampling station	CEDEX category (1994)	Contaminants of concern[a]	Toxic effect[b]	
			Pelagic organisms	Benthic organisms
H1	IIIa	As, Cu	-	+++++
H2	IIIa	As, Cu	+	++++
H3	IIIa	As, Cu	+	+++++
H4	I	--	+	-
C1	IIIb	Cd, Cu, Hg, Pb, Zn, PCBs	-	++
C2	IIIb	Pb, Hg, PCBs	-	+
C3	IIIb	Cd, Cu, Hg, Pb, Zn, PCBs	-	++
C4	IIIa	PCBs	-	+++

[a]Compounds exceeding the corresponding AL2.
[b]Identified based on one or more toxicity for bioassays.

Table 6. Dredged material categories and management requirements according to Spanish recommendations (CEDEX, 1994).

Category	Concentrations	Aquatic disposal	Type of license	Requirements
I	C< AL1	Free aquatic disposal	Normal authorization	-Sedimentological studies and biological effects (physical/mechanic).
II	AL1< C< AL2	Dumping under controlled conditions	Special authorization	-Controlled dumping and justification. -Impact hypothesis. -Environmental control management. -Corrective measures.
III	C> AL2	Dumping under adequate management techniques	Special authorization	-Contaminants sources study and source control measures. -Isolation techniques and justification. -Impact hypothesis. -Environmental control program. -Corrective measures.

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Capítulo 7.

Conclusiones

1) La caracterización físico-química de los materiales de dragado permitió estudiar las propiedades y la contaminación de sedimentos procedentes de puertos españoles. Del total de muestras estudiadas, el 64% serían consideradas materiales de Categoría III, para los que no se considera el vertido al mar y, por lo tanto, deberían ser gestionados mediante recintos de almacenamiento adecuados. Sólo el 12% de las muestras estudiadas serían materiales de Categoría I, considerados aptos para su vertido al mar, mientras que el 24% se clasificaron dentro de la Categoría II de acuerdo a su moderada contaminación. Para esta categoría el uso de las guías químicas según las RRGMMD parece no ser suficiente para evaluar si procede su vertido al mar y se consideran otros estudios para poder establecer su calidad ambiental. El uso del cociente medio de todos los compuestos analizados es una buena medida para la clasificación de los materiales según su contaminación, aunque también pueden ser útiles otras técnicas estadísticas multivariantes de fácil aplicación, como el análisis tipo cluster o el análisis de componentes principales. La aplicación de estas técnicas permite caracterizar la contaminación de los sedimentos e identificar, en algunos casos, la fuente de ésta.

2) Se ha diseñado una batería de bioensayos de toxicidad para la caracterización ecotoxicológica de los materiales de dragado. Esta batería incluía distintos ensayos desarrollados sobre el sedimento en bruto para estudiar posibles efectos en las comunidades bentónicas y otros desarrollados sobre los lixiviados por ser indicativos de los

procesos de resuspensión de los materiales de dragado en la columna de agua. La sensibilidad relativa de cada ensayo es distinta de acuerdo a la distinta fase de exposición a los sedimentos considerada por cada organismo. El ensayo menos sensible de entre los de fase sólida fue el ensayo con juveniles de almeja. Los anfípodos, que viven en el sedimento pero además se alimentan de él, son los organismos bentónicos más sensibles de entre los utilizados. El mayor porcentaje de muestras tóxicas correspondió al ensayo con bacterias -Microtox® SPT-, con casi un 80% de muestras tóxicas identificadas. La sensibilidad de los ensayos en fase sólida fue en orden decreciente anfípodos>erizo>poliquetos>almejas, con un porcentaje de muestras tóxicas identificadas del 70, 50, 25 y 18%, respectivamente. Aunque todos los ensayos finalizaron con éxito y sirvieron para clasificar las muestras según su toxicidad, se han considerado algunos factores de confusión que deben ser correctamente identificados para poder interpretar los resultados.

3) El análisis de los resultados físico-químicos y ecotoxicológicos de los sedimentos mostró una mayor sensibilidad de los ensayos en fase sólida a los compuestos de tipo metálico, especialmente el ensayo con anfípodos. Los otros ensayos aparecen correlacionados sólo con algunos de ellos, dependiendo de las respuestas tóxicas registradas para determinados grupos de muestras. Por el contrario, las respuestas biológicas medidas tras exposición a los lixiviados no parecen guardar ninguna relación con las concentraciones de metales y compuestos orgánicos medidas en sedimento. Aunque estos resultados parezcan contradictorios, son razonables si se tiene en cuenta que se está evaluando la toxicidad de los contaminantes tras la extracción acuosa del sedimento y no el sedimento en su totalidad. En cualquier caso, es significativo que ninguno de los efectos medidos se correlacione con la contaminación de tipo orgánico en los sedimentos. Aunque estos resultados parezcan indicar la ausencia de toxicidad de este tipo de compuestos, todos ellos son considerados prioritarios por lo que parece más razonable pensar que las medidas utilizadas en la batería de ensayos no responden a la presencia de este tipo de contaminantes.

4) De la batería de tests, se seleccionaron cuatro de ellos como los más adecuados para un estudio de la variabilidad interlaboratorio y validar así su uso en distintos laboratorios. Estos fueron, el ensayo de inhibición de la luminiscencia con bacterias, el ensayo con anfípodos, el ensayo con juveniles de almeja y el ensayo con embriones de erizo de mar. Este estudio, que comprendió distintos ejercicios desarrollados en 2003 y 2004, mostró

unos resultados de variabilidad aceptables en los rangos descritos anteriormente en la literatura para este tipo de medidas. Considerando el elevado número de condiciones que podían influir en la variabilidad de los resultados –principalmente distintas especies y protocolos flexibles-, los resultados son satisfactorios para el ensayo de inhibición de la luminiscencia con bacterias, siguiendo el protocolo SPT del aparato comercial Microtox®, el ensayo con embriones del erizo de mar *Paracentrotus lividus*, y el ensayo con anfípodos. Para este último ensayo los resultados permitieron validar el uso de distintas especies de importancia regional usadas en este tipo de ensayos: *Corophium volutator*, *Corophium multisetosum*, *Ampelisca brevicornis* y *Mycrodeutopus gryllotalpa*. El principal factor de variabilidad en los resultados parece ser el tiempo de almacenamiento de los sedimentos, aunque en el caso del ensayo de desarrollo larvario la flexibilidad de los protocolos de obtención y manipulación de los lixiviados, y el criterio de toxicidad utilizado por el operador parecen ser la causa de la variabilidad en los resultados para algunos laboratorios. Según los resultados obtenidos, se propone una guía de calidad biológica para el ensayo Microtox® SPT similar a la guía de canadiense para este ensayo establecida en 1000 mg/L expresado en peso seco. Para el ensayo de desarrollo larvario con embriones de erizo de mar, la guía de calidad biológica establecida es un 25% de larvas anormales. Además, tienen que mostrarse significativamente diferentes ($p<0,05$) a los resultados obtenidos en el control. De forma similar, las muestras con una mortalidad de anfípodos significativamente diferentes respecto al control y con una mortalidad superior al 25% fueron siempre consideradas como tóxicas para este ensayo.

5) Se incorporan nuevos bioensayos de bioacumulación para evaluar la biodisponibilidad de los contaminantes presentes en el sedimento y su uso en la caracterización del material de dragado. Los resultados de los ensayos de bioacumulación en el laboratorio con poliquetos de la especie *Arenicola marina* mostraron acumulación de compuestos de tipo metálico. A concentraciones intermedias, la variabilidad en los resultados es mayor. Aunque se ha identificado el contenido en materia orgánica del sedimento como un factor importante a la hora de determinar la bioacumulación de este tipo de compuestos, es difícil ajustar las concentraciones medidas en el sedimento y las concentraciones en organismo de forma directa. Para los estudios de los contaminantes de tipo orgánico, los resultados mostraron las concentraciones de PCBs y de compuestos orgánicos de Hg más elevadas tras la exposición a las mayores concentraciones en sedimento. Por el contrario, esta tendencia no se observa para los PAHs y las concentraciones medidas en los poliquetos no presentaban

una relación directa con las concentraciones en los sedimentos a los que estaban expuestos. Aunque se han desarrollado modelos para estudiar este tipo de procesos de forma teórica, su uso no se ajusta a los datos obtenidos mediante ensayos de laboratorio y son recomendables en un fase temprana de la evaluación. Los resultados de los estudios de bioacumulación utilizando almejas, muestran tendencias similares a los de poliquetos, aunque con la diferencias asociadas con el mecanismo de alimentación distinto que tienen ambos tipos de organismos.

6) Se propone un método escalonado para caracterizar la calidad ambiental de materiales de dragado procedentes de puertos españoles. Este método sigue las RRGMMD y contempla una primera fase para definir el proyecto y la estrategia de muestreo. La caracterización comprende el análisis de los sedimentos mediante ensayos físico-químicos y su comparación con los NNAA para identificar y clasificar aquellos materiales, así como el uso del ensayo de tipo 'screening' Microtox. Para aquellos materiales que se excedan los NNAA1, el cuadro escalonado prosigue con una tercera fase de caracterización ecotoxicologica compuesta del ensayo de mortalidad con anfípodos y el ensayo de desarrollo larvario con embriones de erizo de mar. En caso de que los materiales de dragado presenten contaminación por compuestos que puedan sufrir bioacumulación o biomagnificación, se recomienda utilizar ensayos específicos. Para ello se pueden combinar guías químicas de calidad de sedimento –si están disponibles-, modelos para estimar de forma teórica la bioacumulación potencial en organismos bentónicos y ensayos de laboratorio que estudian de forma directa el aumento de las concentraciones en organismos bentónicos. En estos casos, el aumento de las concentraciones medidas en dos tipos de organismos, *Arenicola marina* y *Ruditapes phillipinarum* respecto a las concentraciones de éstos expuestos a una estación de referencia puede ser suficiente para la correcta caracterización y gestión de los materiales. Este cuadro de caracterización permite la gestión de los materiales de acuerdo al riesgo químico y biológico y supone una mejora al método tradicional en la reducción de la polución marina gracias a la mejor identificación de materiales peligrosos y/o nocivos.

Epílogo del autor

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[1]Dedo

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